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Prolactin (PRL) and Its Receptor: Actions, Signal Transduction Pathways and Phenotypes Observed in PRL Receptor Knockout Mice

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I. Introduction

PRL and GH, along with placental lactogens (PLs), form a family of hormones that probably result from the duplication of an ancestral gene. It was early in the 20th century that changes in the histology of the anterior pituitary gland of pregnant women were first noted. French researchers were the first to identify a pituitary factor capable of inducing milk secretion in rabbits (1). American scientists made similar observations, and in addition to naming the new pituitary factor prolactin, showed that PRL was able to stimulate the growth of the pigeon crop sac (2). PRL has now been shown to exist in all vertebrates thus far examined.

Because human GH preparations were lactogenic in conventional bioassays, and because early attempts to separate GH and PRL activities failed, there was some question whether a separate PRL existed in humans. There was strong clinical and histological evidence to suggest that the two hormones were present in humans. Finally, human PRL (hPRL) was successfully isolated and purified (3, 4), which led to numerous subsequent pathophysiological studies.

PRL has more actions than all other pituitary hormones combined. The initial step in the action of PRL, like all other hormones, is the binding to a specific membrane receptor, the PRL receptor (PRLR). Similar to the ligand, the PRLR has also been shown to be a member of the same family as the GH receptor and also part of the larger class of receptors, known as the class 1 cytokine receptor superfamily.

In this review we will briefly discuss the structure of PRL and its family members and the fact that PRL is produced at sites outside the pituitary gland (extrapituitary PRL), and thus may act as a hormone, by the classic endocrine pathway, and as a growth factor, neurotransmitter, or immunoregulator, in an autocrine-paracrine fashion. The structural organization of the PRLR and its complex binding and activation will be described, as well as the tissue distribution of the receptor. The original list of 85 different actions of PRL in vertebrates has been expanded to include more than 300 separate functions of this multifaceted hormone. The signal transduction mechanisms activated after the binding of PRL to the receptor will be described. Finally, the phenotypes associated with the knockout of the PRLR gene in mice will be reviewed. Although this approach does not apply to all reported functions of PRL (seasonal actions, species-specific

effects, etc.), in many instances the knockout model is useful to identify actions directly associated with PRL or PL and, by comparison with other gene deletions, suggests which actions have been taken over by another hormone or cytokine.

II. PRL

A. The PRL/GH/PL family

Three decades after PRL was identified, the amino acid sequence of sheep PRL (also referred to as lactogenic hormone or luteotropic hormone depending on its biological properties) was determined and shown to be a protein of 199 amino acids (5). At the end of the 1970's, the rapid development of cloning technology allowed the identification of the nucleotide sequence of PRL cDNAs from several species (6). As anticipated from earlier structural studies, the primary structure of PRL appeared closely related to that of two other hormones, GH, also of pituitary origin, and PL, secreted by mammalian placenta (6–8). Today, genetic (7, 8), structural (6, 9), binding (9), and functional (6, 9) studies of these three hormones, as well as the more recently identified somatolactin and PRL-related proteins, have clearly demonstrated that they all belong to a unique family of proteins.

B. PRL gene and primary structure

The gene encoding hPRL is located on chromosome 6 (10). It is composed of five exons and four introns with an overall length of ~10 kb (11). The hPRL cDNA is composed of 914 nucleotides and contains a 681-nucleotide open reading frame encoding a prehormone of 227 amino acids (aa), including a signal peptide of 28 aa (12). The mature hPRL thus contains 199 aa, with a total molecular mass of ~23 kDa.

PRL is present in all vertebrates, and cDNAs encoding PRL from several species have been isolated and sequenced (6, 12–17). With the exception of fish, all PRLs identified so far are 197–199 aa and contain six cysteines forming three intramolecular disulfide bonds (Cys 4–11, 58–174, and 191–199 in hPRL). Fish PRLs are shorter than mammal PRLs and lack a dozen residues at the N terminus, including the first disulfide bridge (16). In tilapia, two distinct PRLs have been isolated, which differ by their length (11 aa), their composition (69% aa identity), and their biological activities (16). These two PRLs presumably result from gene duplication (16) and are likely to reflect a situation unique to fish. Although the primary structure of PRL is highly conserved within a given class [*e.g.*, bovine and human PRLs share 74% aa identity (14), carp and salmon PRLs 77% (18)], PRL sequences from distantly related species show a high degree of divergence [*e.g.*, carp and human PRLs share only 36% of similarity (18)]. Posttranslational modifications of mature PRL, including glycosylation, phosphorylation, or proteolytic cleavage, have been reported and recently reviewed (17, 19).

On the basis of sequence comparisons of tetrapod hormones, Martial and collaborators confirmed the earlier hypothesis formulated by Niall and colleagues (7) that the genes encoding PRL, GH, and PL are evolved from a common ancestral gene (12, 20) and located the divergence of PRL and GH lineages that occurred some 400 million years ago (12, 13). On the other hand, evolutionary studies including fish hormones suggest that the divergence might have occurred up to 820 million years ago (for discussions, see Ref. 21). Finally, PLs, which are only found in mammals, are believed to have an alternative genetic origin, either the GH lineage (primate PLs) or the PRL lineage (nonprimate PLs) (8, 22).

C. PRL tertiary structure

Secondary structure studies (circular dichroism, etc.) have shown that PRL is an all-α-helix protein and contains almost 50% of α-helices, while the remainder of the protein appears to fold into nonorganized loop structures (23). To date, attempts to determine the three-dimensional (3D) structure of PRL via experimental techniques (x-ray, nuclear magnetic resonance) have been unsuccessful. However, taking advantage of the structure/function similarities between PRL and GH (see above), we have recently determined the 3D structure of hPRL using the homology modeling approach (24) based on the crystallographic coordinates of porcine (p) GH (25). As anticipated, hPRL is predicted to fold in a four-helix bundle and to share with GHs the particular up-up-down-down connectivity of the α-helices (9, 24–26) (Fig. 1A).

D. Extrapituitary PRL

In addition to being synthesized and secreted by lactotrophic cells of the anterior pituitary gland, PRL is also produced by numerous other cells and tissues. The subject of extrapituitary PRL has recently been reviewed (27) and thus will not be described here in detail.

In addition to the anterior pituitary gland, PRL gene expression has been confirmed in various regions of the brain, decidua, myometrium, lacrimal gland, thymus, spleen, circulating lymphocytes, and lymphoid cells of bone marrow, mammary epithelial cells and tumors, skin fibroblasts, and sweat glands (reviewed in Ref. 27). PRL can thus be found in several fluid compartments in addition to serum, such as cerebrospinal fluid, amniotic fluid, tears, milk, follicular fluid, and sweat. Interestingly, hypophysectomized rats retain ~20% of biologically active PRL in the circulation, which increases to ~50% of normal levels with time. Neutralization of circulating PRL with anti-PRL antibodies results in immune dysfunction and death (28), suggesting that extrapituitary PRL is important and, under some circumstances, can compensate for pituitary PRL.

Pituitary PRL acts via a classic endocrine pathway, *i.e.*, it is secreted by a gland, transported by the circulatory system, and acts on target cells at some peripheral sites via specific receptors located on the plasma membrane. The PRL that is produced by many different cell types can act in a more direct fashion, *i.e.*, as a growth factor, neurotransmitter, or immunomodulator, in an autocrine or paracrine manner. Thus, locally produced PRL can act on adjacent cells (paracrine) or on the PRL-secreting cell itself (autocrine). Using paracrine or autocrine mechanisms, it would thus be possible to activate many of the actions associated with PRL without ever affecting the circulating concentration of the hormone.

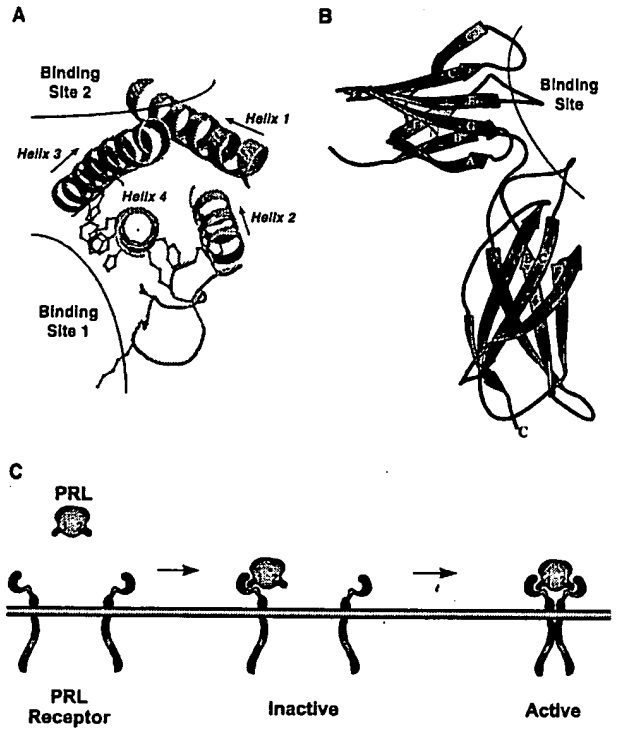


FIG. 1. A, Ribbon representation of the predicted 3D structure of hPRL, modeled on the basis of the crystallographic structure of porcine GH (24, 25). hPRL is predicted to adopt the four-helix bundle folding described for GHs (24, 26, 77). Location of binding sites 1 and 2 (see text) is indicated. Side chains of amino acids involved in binding site 1, as deduced from mutational studies (9), are represented. B, Ribbon representation of the 3D x-ray structure of a monomer of the human PRLR ECD (62). The ECD folds in a β-sandwich formed by two antiparallel β-sheets (see text). N- and C-terminal ends are indicated by N and C, respectively. This figure was kindly provided by Drs. P. Elkins and A. M. de Vos. Note that the structures depicted in panels A and B are not at the same scale (see Ref. 62). C, PRLR activation by PRL-induced dimerization. Hormone binding to PRLR is sequential. First, the hormone (H) interacts with the receptor (R) through its binding site 1 (see Fig. 1A), forming an inactive H_1:R_1 complex. Then, the hormone binds to a second receptor through its site 2, which leads to receptor homodimerization and formation of an active H_1:R_2 complex. Hormone analogs whose binding site 2 is sterically blocked are unable to induce receptor homodimerization and are thus inactive; since they still bind to the receptor through site 1, they behave as antagonists of wild-type hormones (9).

III. PRL Receptor (PRLR)

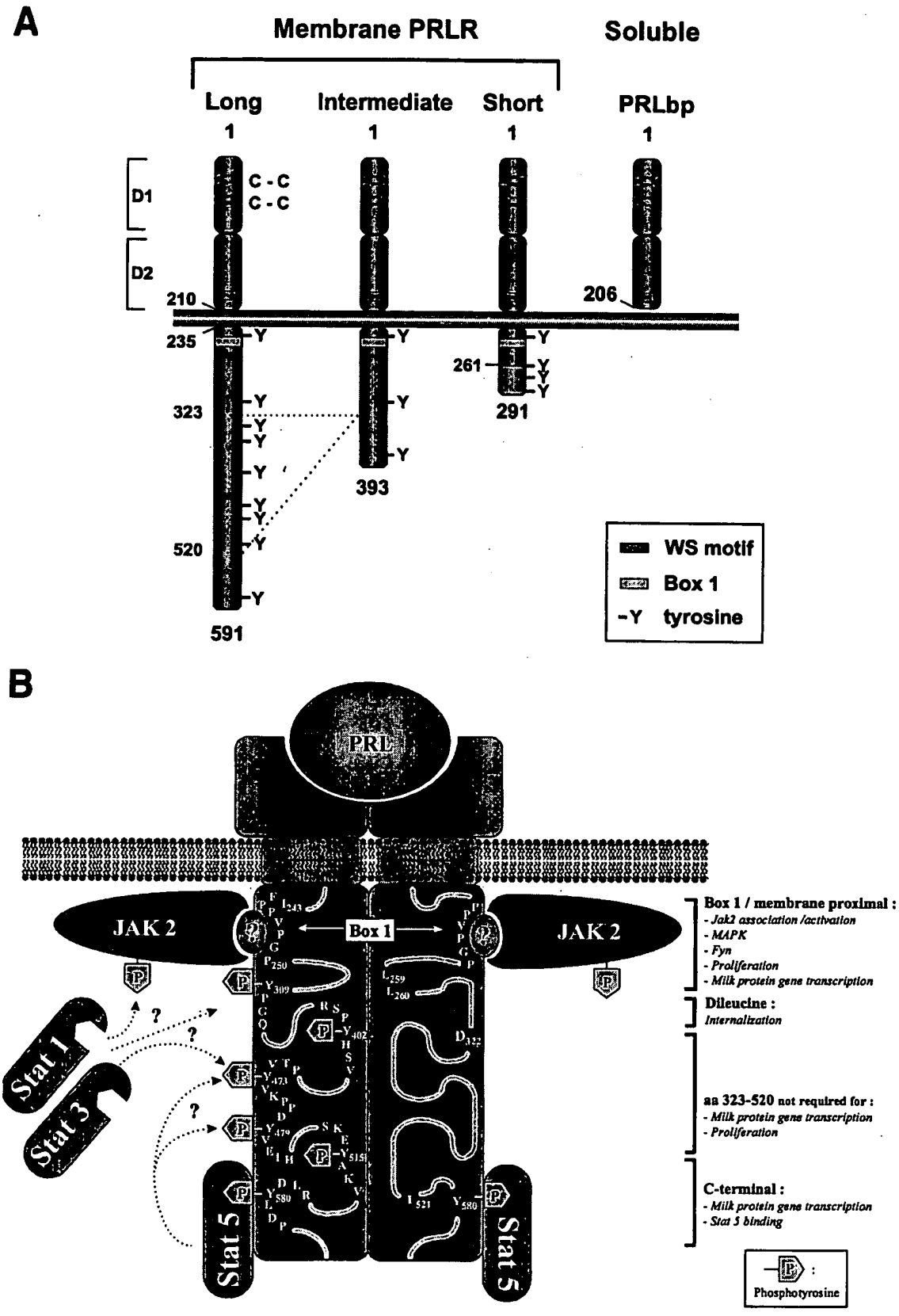
A. The class 1 cytokine receptor superfamily

More than two decades ago, the PRLR was identified as a specific, high-affinity, membrane-anchored protein (29–32). In 1988, the cDNA encoding the rat PRLR was isolated in our laboratory (33) and, as is true for their respective ligands, receptors for PRL and GH (GHR) are also closely related (33–35). Both are single-pass transmembrane chains and, despite a relatively low degree (~30%) of sequence identity, they share several structural and functional features (35–38). In the early 1990's, sequence comparison with newly identified membrane receptors led to the identification of a new family of receptors including both PRLR and GHR (35, 39, 40). Termed class 1 cytokine receptors, this superfamily includes receptors for several interleukins, granulocyte-colony stimulating factor (G-CSF), granulocyte macrophage-colony stimulating factor (GM-CSF), leukemia inhibitory factor (LIF), Oncostatin M (OM), erythropoietin (EPO), thrombopoietin (TPO), gp130, and the obesity factor leptin (41–45). Although all these membrane chains are apparently genetically unrelated, they contain stretches of highly conserved amino acids, both in the extracellular and the intracellular domains. These conserved features are described below with respect to the PRLR.

B. PRLR gene and primary structure

The gene encoding human PRLR is located on chromosome 5 (p13→14) and contains at least 10 exons for an overall length exceeding 100 kb (46). Contrary to PRL, for which a single transcript encodes a unique mature protein, multiple isoforms of membrane-bound PRLR resulting from alternative splicing of the primary transcript have been identified in several species (33, 47–51). These different PRLR isoforms differ in the length and composition of their cytoplasmic tail and are referred to as short, intermediate, or long PRLR with respect to their size (Fig. 2A) (For review, see Refs. 35, 37, and 38). For example, in rat, the PRLR isoforms contain 291 (short), 393 (intermediate), or 591 (long) aa. In mice, one long and three short isoforms have been identified, the short forms only differing by a few amino acids in the C-terminal part of their cytoplasmic tail (51, 52). In addition to the membrane-anchored PRLR, soluble isoforms have also been identified (PRL binding protein, or PRLbp), but whether they result from alternative splicing of the primary mRNA or proteolytic cleavage of membrane-bound PRLR (or both) is uncertain (53–55). In all cases, however, the extracellular, ligand-binding domain is identical, whatever the isoform. Detailed description of the various PRLR isoforms from different species has been provided in previous reviews (35, 37, 38, 56), and this aspect is thus not developed in this review, except when required.

1. The extracellular domain (ECD). Most of the sequence similarities between cytokine receptors are found within their ECD. Typically, a cytokine ECD is composed of a domain of ~200 aa, referred to as the cytokine receptor homology (CRH) region (45). The CRH can be divided into two subdomains of ~100 aa (referred to as D1 and D2), each showing analogies with the fibronectin type III module (35, 40, 45). Although some cytokine receptors contain additional domains, it seems that ligand interactions are primarily driven by the conserved fibronectin-like domains (45, 57). In rat and human, the PRLR ECD encompasses the 210 amino-terminal residues, whatever the isoform considered (33, 35, 48). Two highly conserved features are found in the cytokine receptor ECDs: the first is two pairs of disulfide-linked cysteines in the N-terminal subdomain D1 (Cys12-Cys22 and Cys51-Cys62 in hPRLR), and the second is a pentapeptide termed "WS motif" (Trp-Ser-any amino acid-Trp-Ser) found in the membrane-proximal region of the C-terminal subdomain D2 (Fig. 2A). The functional importance of these features is discussed below (*Section III.D*). The case of avian PRLRs is atypical since, at least in pigeon and chicken, the PRLR ECD is du-


A
Membrane PRLR
Soluble
Long
Intermediate
Short
PRLbp
1
1
1
1
D1
D2
C - C
C - C
210
206
235
261
323
291
393
520
591
Y
WS motif
Box 1
-Y tyrosine
B
PRL
JAK 2
JAK 2
Box 1
Stat 1
Stat 3
Stat 5
Stat 5
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?
?
Box 1 / membrane proximal :
- Jak2 association /activation
- MAPK
- Fyn
- Proliferation
- Milk protein gene transcription
Dileucine :
Internalization
aa 323-520 not required for :
- Milk protein gene transcription
- Proliferation
C-terminal :
- Milk protein gene transcription
- Stat 5 binding
P :
Phosphotyrosine

plicated and contains two highly homologous CRH regions (58). The additional N-terminal module does not seem to play any functional role since its deletion has no significant effect on the ligand-binding affinity, ligand specificity (58), or signal transduction (59) of the pigeon PRLR.

2. *The transmembrane domain.* Like all cytokine receptors, the PRLR is a single-pass transmembrane chain. The transmembrane domain is 24 aa long (aa 211–234 in rat PRLR). The involvement of this region (or of any crucial amino acid within this domain) in the functional activity of the receptor is unknown.

3. *The intracellular domain.* The cytoplasmic domain of cytokine receptors displays more restricted sequence similarity than the ECD. Two regions, called box 1 and box 2 (35, 60), are relatively conserved. Box 1 is a membrane-proximal region composed of 8 aa highly enriched in prolines and hydrophobic residues (aa 243–250 in PRLR; Fig. 2A). Due to the particular structural properties of proline residues, the conserved P-x-P (x = any amino acid) motif within box 1 is assumed to adopt the consensus folding specifically recognized by transducing molecules (see below). The second consensus region, box 2, is much less conserved than box 1 and consists in the succession of hydrophobic, negatively charged, then positively charged residues (aa 288–298). While box 1 is conserved in all membrane PRLR isoforms, box 2 is not found in short isoforms (35, 38).

In a recent study (61), we have identified, within the cytoplasmic domain of the short PRLR, two motifs required for receptor internalization. The first involves a dileucine motif (aa 259–260); the second contains a tetrapeptide predicted to fold in a β-turn (aa 276–279). Interestingly, the long PRLR isoform, which is less efficiently internalized than the short form, lacks the putative β-turn motif (61).

C. PRLR tertiary structure

The 3D structure of genetically engineered hPRLR ECD (*i.e.*, hPRLbp) has been determined by crystallographic analysis (62) (Fig. 1B). Each fibronectin-like subdomain (D1 and D2) contains seven β-strands that fold in a sandwich formed by two antiparallel β-sheets, one composed of three strands referred to as strands A, B, and E, and the other composed of the four remaining strands termed C, C′, F, and G (26, 38, 45, 57, 62). Both subdomains are linked by a small four-residue polypeptide (26, 62). As anticipated from sequence comparison (40), this folding pattern is likely to be shared by several, if not all, cytokine receptors, since it has also been described for the ECDs of the hGH receptor (26) and the EPO receptor (63, 64) as well as for the α-chain of the interferon (IFN)-γ receptor, a class 2 cytokine receptor (65). To the best of our knowledge, no structural data have been reported yet for the cytoplasmic domain of any cytokine receptor, including the PRLR.

D. PRLR binding and activation by PRL

No exhaustive information on the amino acids of the PRLR ECD interacting with PRL is yet available. Actually, two mutational studies performed in our laboratory focused on some features conserved in cytokine receptor ECDs, including the two pairs of disulfide-bonded cysteines and the WS motif (see above, *Section III.B*) (66, 67). In agreement with similar studies performed on the GHR (68), mutation of any of these conserved cysteines leads to impaired structural and functional properties of the receptors (66), although only amino acids bordering the first, but not the second, disulfide bridge are likely involved in ligand binding (67). Despite the fact that structural data clearly indicate that the WS motif in both GHR (26) and PRLR (62) is located away from the ligand-binding interface, mutations within this conserved feature are detrimental to binding affinity (67, 69) (for review, see Ref. 38). Actually, functional studies of several cytokine receptors (69–72) have suggested that the WS motif is probably required for correct folding and cellular trafficking rather than for ligand binding itself (for discussions, see Ref. 38). Finally, in addition to these features typically conserved in cytokine receptors, we have also suggested that two tryptophans (Trp72 and Trp139) of the PRLR are involved in PRL binding. This hypothesis is consistent with the 3D structure of the two homologous complexes, hGH-hGHbp and hGH-hPRLbp (26, 62), and suggests that these two tryptophans represent a specific feature of the ligand-receptor interactions within the PRL/GH family (24, 38). The three asparagine-linked glycosylation sites present in the ECD of the PRLR do not appear to be involved in ligand binding (62, 73, 73a).

Although stoichiometric analysis of the interaction between different PRLR ECDs and lactogenic hormones achieved 1:1 (74) or 1:2 (75) complexes depending on the species involved, dimerization of the PRLR upon ligand binding has now been clearly established after different approaches. First, we have shown that at least two regions of

FIG. 2. A, Schematic representation of soluble (human) and membrane (rat) isoforms of the PRLR (33, 47, 50, 54). Although the mechanism of PRLbp generation remains unclear (alternative splicing or proteolysis or both), an mRNA encoding a soluble PRLbp of 206 aa has been isolated in the human breast cancer cell line BT-474 (54). All forms have identical extracellular, ligand-binding domains. Subdomain D1 contains two pairs of disulfide bonded-cysteines (C-C) and subdomain D2 contains the WS motif (*green box*), two characteristic features of the cytokine receptor superfamily. Box 1 (*orange box*) is found in the cytoplasmic domain of all membrane isoforms. In rat, the intermediate PRLR (only found in Nb2 cells) differs from the long isoform by a 198-aa deletion in the cytoplasmic domain (aa 323–520). Otherwise, the short PRLR is identical to both other isoforms up to residue 261, after which its sequence differs (*light blue box*). Cytoplasmic tyrosine residues are indicated. B, Structure-function relationships of the long PRLR cytoplasmic domain. Box 1 is required for JAK2 binding; whether this interaction is direct or mediated by an adapter is unknown. The di-leucine motif (aa 259–260), identified in the short PRLR, is presumably involved in internalization of all PRLR isoforms. Six tyrosines (of the nine present in rat PRLR) are potentially phosphorylated. The most C-terminal (Y580), required for Stat5 activation, is proposed to be the major binding site of this Stat protein. Y479 and Y473 can also activate Stat5, although to a lesser extent; these may be Stat5-binding sites of lower affinity. Stats 1 and 3 are likely to interact with membrane-proximal regions of the receptor complex; candidates are Y309 on the receptor or tyrosines to be defined within JAK2. The membrane-proximal region that is common to all PRLR isoforms is required for interaction with and/or activation of JAK2, Fyn, and MAP kinases as well as for activation of cell proliferation and transcription of milk protein genes.

hPRL are involved in the binding of the hormone to the PRLR. The first, referred to as binding site 1, encompasses several residues belonging to helices 1 and 4 (24, 76–78), while the second, termed binding site 2, involves helices 1 and 3 (24, 79, 80) (Fig. 1A). Detailed analysis of individual residues required for tight receptor binding has been reported in a recent issue of *Endocrine Reviews* (9). Second, analysis of PRLR ECD-lactogenic hormone complexes using surface plasmon resonance technology (BIAcore, Pharmacia & Upjohn AB, Stockholm, Sweden) has demonstrated the formation of 1:2 complexes (81). It is likely that the very rapid dissociation of 1:2 complexes for 1:1 complexes has prevented their identification by classic gel filtration experiments (74), as well as by the two-hybrid approach (82). Third, elucidation of the events occurring upon PRL-induced activation of membrane-bound PRLR has resulted from the close analysis of the shape of experimental curves obtained with point-mutated hPRL analogs in PRL-responsive bioassays performed over a wide range of hormone concentrations (reviewed in Ref. 9). As first described for the closely related GHR (45, 83, 84), activation of the PRLR involves ligand-induced sequential receptor dimerization (Fig. 1C). In a first step, interaction of PRL binding site 1 with one PRLR occurs and leads to the formation of an inactive H_1:R_1 (one hormone, one receptor) complex. Formation of this complex appears to be a prerequisite for PRL binding site 2 to interact with another PRLR, thereby achieving an active trimeric complex (H_1:R_2), composed of one hormone and one receptor homodimer. In agreement with this model, hPRL analogs carrying a disruptive mutation in binding site 2 are inactive (since they are unable to induce PRLR dimerization) but display antagonistic properties due to their ability to block the receptor in the inactive H_1:R_1 stoichiometry (9, 37, 80).

IV. Distribution of the PRLRs

PRL-binding sites or receptors have been identified in a number of cells and tissues of adult mammals. The expression of short and long forms of receptor have been shown to vary as a function of the stage of the estrous cycle, pregnancy, and lactation (85–88). As can be seen in Table 1, PRL-binding sites or receptors are widely distributed throughout vertebrates. There was, however, very little information on the expression of this receptor during fetal development. To that end, we have recently determined the cellular distribution and developmental expression of the PRLR in the late gestational fetal rat by *in situ* hybridization, immunocytochemistry, and radioligand binding (89). Sense and antisense strand probes were prepared encoding the long and short isoforms of the rat PRLR and hybridized to various fetal tissues obtained at the end of pregnancy (days 17.5 to 20.5). These studies showed that the mRNA encoding the short and long isoforms was widely expressed in tissues from all three germ layers: in addition to the classic target organs of PRL, tissues not known previously to contain PRLRs, such as olfactory neuronal epithelium and bulb, trigeminal and dorsal root ganglia, cochlear duct, brown adipose tissue, submandibular glands, whisker follicles, tooth primordia, and proliferative and maturing chondrocytes of developing bone, also expressed PRLR. There was also a high level of expression of receptor mRNA in the fetal adrenal cortex, gastrointestinal and bronchial mucosae, renal tubular epithelia, choroid plexus, thymus, liver, pancreas, and epidermis.

To complement the *in situ* studies, immunohistochemical studies using monoclonal anti-PRLR antibodies clearly demonstrated the distribution of PRLR immunoreactivity was similar to that of the mRNA, strongly suggesting that the receptor protein is expressed in the developing fetus. The functional activity of the PRLRs was established by the demonstration of specific rat PL II binding sites in fetal adrenal cortex, renal tubules, small intestinal villi, pancreatic ductules and islets, hepatic parenchyma cells, choroid plexus

TABLE 1. Distribution of PRL binding sites in vertebrates

Cell or tissue	Cell or tissue
Central nervous system	Kidney
Brain	Cortex
Cortex	Bladder (fish, reptiles, amphibians)
Hippocampus	Lymphoid tissue
Choroid plexus	Spleen
Striatum	Thymus
Cochlear duct	Nurse cells
Corpus callosum	Epithelial cells
Hypothalamus	Lymphocytes
Astrocytes	T
Glial cells	B
Retina	Macrophages
Olfactory system	Ganglia
Ganglia	Intestinal cells
Pituitary	Reproductive system
Anterior lobe	Female
Intermediate lobe	Ovary
Adrenal cortex	Ova
Skin	Granulosa cells
Epidermis	Thecal cells
Hair follicle	Corpus luteum (luteal cells)
Sweat gland	Oviduct
Bone tissue	Mammary gland
Chondrocytes	Epithelial cells
Cartilage	Milk[a]
Osteoblasts	Tumors
Gills (fish and larval amphibians)	Crop sac (birds)
Lung	Uterus (endometrium)
Heart	Placenta
Cardiac muscle	Amnion
Atria	Male
Skeletal muscle	Testis
Adipocytes (birds)	Germ cells
Brown adipose tissue	Spermatozoa
Liver	Leydig cells
Hepatocytes	Sertoli cells
Kupffer cells	Epididymis
Submandibular gland	Seminal vesicle
Submaxillary gland	Prostate
Pancreas	
Islet of Langerhans	
Gastrointestinal tract	
Esophagus	
Stomach	
Intestine	
Duodenum	
Jejunum	
Ileum	
Colon	

[a] Membrane and soluble forms.

ependymal cells, fetal lung, and thymus. The level of PRLR mRNA and protein actually increased between days 17.5 and 20.5 of pregnancy in a number of tissues, including the adrenal, pancreas, small intestine, pituitary, thymus, liver, and submandibular gland. These results suggest that lactogenic hormones such as PRL and PLs may play important roles in fetal and neonatal development (89).

Since the PRLR is expressed at relatively low levels in the olfactory bulb of the adult rat, but is easily detected in late pregnancy in the fetal rat, we decided to investigate the ontogenesis of PRLR expression in the olfactory system, again using *in situ* hybridization and immunohistochemistry (90). At embryonic day 12.5 (e12.5), mRNAs encoding the long and short isoforms of the PRLR were detected in the medial and lateral nasal processes, the epithelial lining of the olfactory pit, and the neuroepithelium lining of the cerebral ventricles, in the region of the rhinencephalon. PRLR mRNA was also highly expressed in the frontonasal mesenchyme and the mesenchymal tissue underlying the developing brain and in the interpeduncular fossa. Once again, the PRLR immunoreactivity was similar to that of mRNA, suggesting that the PRLR gene was translated in lactogen binding sites or receptors in the developing embryo. As pregnancy advanced, the receptor was expressed intensely, albeit discontinuously, in the olfactory system. Receptor expression was also seen in the cartilage primordia of the ethmoid, sphenoid, temporal, and mandibular bones. Although the PRLR was expressed in the vomeronasal organ, it was limited to the luminal epithelial surface.

It was not until embryonic day 18 that PRLR mRNA and protein was detected in the olfactory bulb. The highest level of expression was seen in the periventricular neuroepithelium. Thereafter, strong staining was observed in the mitral and tufted cell neurons and the sensory neuronal cell bodies of the olfactory epithelium. This high level of expression continued until neonatal day 5. Interestingly, PRLR expression was also found in the mitral cells of the olfactory bulb of the lactating rat, although the levels appear to be much lower than those seen in the fetal and neonatal rat. These studies suggest novel roles for lactogenic hormones in olfactory differentiation and development and may provide new mechanisms by which lactogenic hormones may regulate neonatal behavior and maternal-infant interactions (90).

V. Biological Functions of PRL

PRL was originally isolated by its ability to stimulate mammary development and lactation in rabbits and soon thereafter to stimulate the production of crop milk in pigeons (1, 2). PRL was shown also to be luteotrophic, that is to promote the formation and action of the corpus luteum (91). Subsequently, a number of additional activities have been associated with this hormone in various vertebrate species. In the now classic reviews by Nicoll and Bern (92) and Nicoll (93), 85 different biological functions of PRL were subdivided into five broad categories: 1) reproduction, 2) osmoregulation, 3) growth, 4) integument, and 5) synergism with steroids. Although these reviews were not exhaustive, the authors tried to consider only relevant effects and disregard those of uncertain validity. This elevated number of biological actions associated with PRL exceeded by far all of the reported actions of the other anterior pituitary hormones combined.

Since the publication of these reviews in 1972 and 1974, numerous other biological functions of PRL have been identified. This section attempts to deal with the now classic functions of PRL and incorporate the more recent findings in the compilation of the actions of this multifaceted hormone. In addition, we decided to modify the categories originally reported, since there was some overlap, and more importantly, the section on reproduction was, in our estimation, too vast. We have thus divided the actions of PRL into the following categories: 1) water and electrolyte balance, 2) growth and development, 3) endocrinology and metabolism, 4) brain and behavior, 5) reproduction, and 6) immunoregulation and protection. We have described actions and cited references dealing with especially well known actions of PRL in lower species, even though these actions may not be seen in mammals. Such actions of PRL may have been lost with evolution or may only be seen in higher animals during certain stages of development. In addition, certain actions of pituitary-derived PRL (endocrine) may be taken over by locally produced PRL (autocrine or paracrine, see *Section II.D*).

A. Water and electrolyte balance

Regulation of salt and water balance is an essential aspect of homeostasis for most organisms. This is especially true for animals living in environments that desiccate them (land or seawater) or that inundate them with water or leach out salts (fresh water). PRL is clearly involved in water and electrolyte balance in almost all classes of vertebrates, although these effects are more difficult to demonstrate in mammals, in spite of the fact that PRLRs are present in kidney, as well as other tissues involved in salt balance.

Osmoregulatory problems are greatest for fish that migrate between fresh and seawater. In a marine environment, fish drink large amounts of water to replace water lost osmotically through the gills. They absorb ions from seawater and excrete them by active transport through the gills. Thus, marine fish can absorb water from the gut and replace what is lost. Fish excrete a minimal volume of urine (93).

In fresh water, the body fluids are hypertonic to the external environment and thus must cope with the problem of water inundation and the loss of ions that diffuse out through the gills. Adaptation to the freshwater habitat necessitates a reduction of gill permeability to salt loss and changes the transport process from active excretion to active uptake. The kidney eliminates the increased water by increasing the rate of urine flow (93).

As depicted in Table 2, PRL plays a major role in regulating water and electrolyte balance through the gill and kidney of many fish and has been referred to as a fresh water-adapting hormone. PRL clearly reduces Na^+ loss or efflux (94) and water uptake or permeability (95) in the gill and increases extracellular volume (104) in the kidney. Na^+ reabsorption is also enhanced in the bladder of fish and amphibians (106, 107). Electrolyte changes and water drive are other effects of PRL in amphibians (109, 110). Birds eliminate excess salt by increasing secretion of the nasal salt gland, which is also

TABLE 2. Water and electrolyte balance

Organ or Target	Effect	Animal class	Reference
Gills	↓ Na^+ loss (efflux)	Fish	(94)
	↓ Water uptake (permeability)		(95)
	↑ Mucus cell size and number		(96)
	Inhibition of Na^+-K^+-ATPase		(97)
	Stimulation of Ca^{2+}-ATPase		(98)
	↓ Ca^{2+} loss (efflux)		(99)
Kidney	↑ Size of renal glomeruli	Fish	(100)
	Hypertrophy of renal tubular cells		(101)
	Stimulation of Na^+-K^+-ATPase	Fish, mammals	(97, 102)
	↓ Na^+ and K^+ excretion	Mammals	(103)
	↑ Extracellular volume	Amphibians	(104)
	↑ Plasma urate	Birds	(105)
	↑ Urine flow rate		(105)
Bladder	↑ Na^+ reabsorption	Fish, amphibians	(106, 107)
Skin	↑ Mucus cell size and number	Fish	(108)
	Electrolyte change and water drive	Amphibians	(109, 110)
Sweat gland	↓ Na^+ and Cl^- in sweat	Mammals	(111)
Intestine	↑ Water and salt absorption:	Mammals	(112)
	Duodenum: Na^+, Ca^{2+}		(113)
	Jejunum: Na^+, K^+, Ca^{2+}		(114)
	Ileum: Na^+, Cl^-, K^+, Ca^{2+}		(115)
	Colon: Na^+, Cl^-,		(116)
Nasal salt gland	↑ Secretión (elimination of excess salt)	Birds	(117)
Uterus	↑ Ca^{2+}, Na^+, K^+, Cl^- in flushings	Mammals	(118)
Placenta	↓ Fluid volume in amnion	Mammals	(119)
	Pathology		
Placenta	Polyhydramnios	Mammals	(120)
Sweat gland	Cystic fibrosis	Mammals	(121)

stimulated by PRL (117). Although the effects on salt and water balance are much less clear than in fish and amphibians, in mammals PRL has been shown to reduce renal Na^+ and K^+ excretion (103) and to stimulate Na^+-K^+ adenosine triphosphatase (ATPase) (102). In addition, PRL decreases Na^+ and Cl^- in sweat (111) and increases water and salt absorption in all regions of the intestine (112). Finally, PRL induces a reduction in fluid volume in the amnion (119).

B. Growth and development

A large number of the reported effects of PRL are associated with growth and development. Many of these are seen in lower vertebrates, but more recent data confirm that cellular proliferation is also one of the important functions of PRL in mammals. The varied functions associated with the category are summarized in Table 3.

1. Body growth. Although PRL and GH are produced by cells of the anterior pituitary that have a common stem cell, there are clear and distinct functions of these two hormones. Thus, in humans that lack GH or do not have a functional GHR, dwarfism is always observed, in spite of the fact that the pituitary produces a functionally active PRL. There have been isolated reports of a somatogenic effect of PRL on body weight in male rats (123) and a small effect on postnatal body growth (124), but most investigators fail to see any consistent effect on overall body size. Finally, in mice lacking the PRLR gene, and thus any functional PRLR (see *Section VII*), there is no modification in overall body length (214), which argues strongly against a direct effect of PRL on body growth.

Interestingly, Ames dwarf mice lacking GH, PRL, and TSH have a longer lifespan than normal mice, in spite of the fact that these animals show some characteristics of reduced immune function (215). Whether this extended life is due to deficiency of the GH/insulin-like growth factor-I (IGF-I) pathway or to PRL deficiency remains to be investigated. The PRLR knockout mouse (see *Section VII*) represents a good model by which to determine whether the PRL pathway is directly involved in aging (214).

There appears to be extensive overlap in many of the biological functions of PRLs and GHs in many lower vertebrates. In birds, for example, an increase in body weight has been observed in PRL-treated males that was significantly higher than that of control animals (122).

2. Growth and metamorphosis. In amphibians, PRL is best known for its antimetamorphic effects. In larvae, PRL has been shown to increase the growth of gills and caudal fin (127, 128) and to increase tail length (126, 129) and is thus considered to be a larval GH. At the premetamorphic stages, PRL reduces growth of the hind legs (130, 131) and prevents resorption of the tail (132–134), both effects being mediated by a reduction in thyroid hormone receptor autoinduction. Some species of fish also undergo metamorphosis. For example, in the Japanese flounder, injections of ovine PRL antagonized the stimulatory effect of T_3 on the resorption of the dorsal fin rays, whereas ovine GH had no effect (125). Finally, in amphibians, PRL induces a metamorphosis of sodium channels (139, 140) and, in mammals, of visual pigments in the retina (200).

3. Cell proliferation. As seen in Table 3, many of the actions of PRL are associated with an effect on cell proliferation and development. One of the major targets is skin. In reptiles and amphibians, PRL promotes molting of the epidermis (135).

PRL stimulates skin melanocyte growth in fishes and mammals (142, 143), and keratinocyte growth in mammals (144). In birds, PRL induces defeathering and epidermal growth of the incubation patch (136, 141). Finally, in deer and goats, PRL is responsible for seasonal changes in pelage growth cycles (145, 146).

Proliferation of the epithelial cells of the crop sac in pigeons was one of the early functions associated with PRL, and crop sac growth still remains the official biological assay of this hormone (147, 148).

Although the liver is not an organ that undergoes rapid proliferation, there have been some reports suggesting that PRL plays an important role in the turnover of hepatocytes. Initial studies showed that there was a striking increase in the number of mitotic figures in livers of transgenic mice expressing hGH, which binds equally well to PRL and GH receptors. To determine whether the observed effect was due to the somatogenic or lactogenic activity of hGH, hepatocytes were isolated from 20-day-old rats and cultured in the presence of rat GH or rat PRL. Interestingly, these cells from young animals, in contrast to normal hepatocytes from adult animals, proliferate in culture. Rat PRL was able to significantly increase the number of mitotic figures in these cells, in comparison to rat GH, which actually slightly decreased proliferation (154). This suggests a functional role for PRL in hepatocyte growth, at some time during development.

Although for many years no clear function could be associated with the presence of the relatively high expression of PRLRs in liver, examination of Table 3 reveals that there are now a number of factors activated by PRL in liver: these include protein kinase C (PKC), diacylglycerol, mitogen-activated protein (MAP) kinase, and phosphoinositide turnover (155–158). In addition, several growth-related genes are induced in liver by PRL, such as IGF-I, ornithine decarboxylase (ODC), *c-myc*, *c-fos*, *c-jun*, and *c-src* (153, 156, 159, 162). Although IGF-I is normally associated with GH stimulation, there may be some instances in which the expression of this growth factor is more responsive to PRL. The ODC gene is also induced and the protein activated in a number of other tissues, including heart, kidney, muscle, adrenal, gonads, and prostate (153, 178, 192, 195).

The proliferation of many cells not normally associated with a direct effect of PRL has also been reported. This hormone has been shown to induce an increase in the size of the intestinal mucosa (171, 172) and proliferation of vascular smooth muscle (176, 177), of β-cells of the pancreas (180–182), of pituitary GH3 cells (187), of human benign prostate hypertrophy epithelial cells (193), of astrocytes (198), and of various cells of the immune system (202, 203).

4. *Differentiation and development.* PRL has also been suggested to have some functional activity in various developmental processes. In addition to its antimetamorphic properties, PRL induces maturation of the lung and surfactant production (149, 152), differentiation of preadipocytes (179), maturation of germ cells (188, 189), and tuberoinfundibular hypothalamic dopamine development (199).

5. *Tumor growth.* Finally, PRL has been associated with certain forms of tumors and may be directly or indirectly involved in tumor growth. These observations are summarized in *Section V.G* on actions associated with pathological disease states. Interestingly, the Moloney murine leukemia virus integration-2 locus colocalizes to the same region of chromosome 2 (rat) and 15 (mouse) as the PRLR and GHR. In one rat T cell lymphoma line, the PRLR gene was activated by provirus integration into the PRLR promoter, rendering the lymphoma sensitive to PRL (210).

C. Endocrinology and metabolism

The endocrine and metabolic effects of PRL not related to reproduction are summarized in Table 4. PRL has been shown to effect energy metabolism by modulating ATPase activity in monkey brain: Na^+-K^+-dependent ATPase was stimulated while Mg^{2+} and Ca^{2+}-dependent ATPases were reduced in neural as well as glial cells (216).

PRL has marked effects on lipid metabolism. In birds, it augments lipoprotein lipase activity in adipocytes (221), although this effect is not seen in mammals, as the adipocyte does not have PRLRs. In mammals, PRL stimulates phospholipid synthesis in the fetal lung (149) and lipoprotein lipase activity in liver (217). Endogenous and exogenous organic compounds, such as cholesterol, bile salts, drugs, and metabolites that cannot be handled by the kidney, are secreted into bile and eliminated. The active transport of bile acids from the plasma to the bile canaliculus, followed by passive movement of water, is an important determinant of bile flow and one of the major hepatic functions. The transport of bile acids such as taurocholate across the basolateral plasma membrane involves a Na^+-K^+ ATPase. PRL has been shown to increase bile secretion (218) and the mRNA encoding the Na^+-taurocholate cotransport polypeptide and hepatic Na^+-taurocholate cotransport (219, 220).

PRL has been reported to affect carbohydrate metabolism in several vertebrate classes, including hyperglycemic/diabetogenic actions. PRL has a differential effect on the activities of enzymes involved in the Embden-Meyerhoff pathway and the hexose monophosphate shunt in neural and glial cells of male monkeys (222). In addition, PRL at physiological concentrations produced a 4-fold increase in glycogen phosphorylase-a activation in isolated hepatocytes (223). Finally, PRL is known to have direct effects on pancreatic function, increasing insulin secretion (185, 224, 225), decreasing glucose threshold for insulin secretion (226), and increasing glucokinase and glucose transporter 2 (227).

A direct action of PRL on adrenal steroidogenesis has been reported. Specifically, PRL is reported to increase adrenal androgens, dihydroepiandrosterone and dihydroepianstrosterone sulfate (229) as well as cortisol and aldosterone (228). Interestingly, a stimulatory effect on 21-hydroxylase activity has also been reported (230). Although there are a number of reports in the literature suggesting that PRL is able to stimulate adrenal catecholamine synthesis directly (234, 235), since there are no receptors in adrenal medullary cells (see Table 1), the effect may be mediated via an increased secretion of adrenocortical hormones stimulated by PRL. In skin, PRL has been shown to increase the expression of type IV 3β-hydroxysteroid dehydrogenase (231).

Although PRL signal transduction is covered in another

TABLE 3. Growth and development

Organ or target	Effect	Animal class	Reference
Body	↑ Weight	Birds, mammals	(122, 123)
	↑ Postnatal body growth	Mammals	(124)
Dorsal fin	Resorption	Fish	(125)
	Larvae		
Gill	↑ Growth	Amphibians	(126)
Tail fin	↑ Growth-via ↑ collagen synthesis		(127, 128)
	↑ Length		(126,129)
	Premetamorphosis		
Hind legs	↓ Growth		(130, 131)
	↓ T_3 receptor autoinduction		(132)
Tail fin	Prevents loss of tail		(133–134)
	↓ T_3 receptor autoinduction		(134)
Tail	Regeneration	Reptiles	(93)
Epidermis	Molting	Reptiles, amphibians	(135)
Feather	Growth	Birds	(93)
	Molting		(136–138)
Skin	Metamorphosis-Na channels	Amphibians	(139, 140)
	Incubation patch-defeathering	Birds	(136)
	Incubation patch-epidermal growth	Birds	(141)
	Proliferation of melanocytes	Fish, mammals	(142,143)
	Proliferation of keratinocytes	Mammals	(144)
	Hair loss for nest building	Mammals (rabbit)	(93)
Hair follicle	Hair growth	Mammals (deer, goat)	(145, 146)
Crop sac	Proliferation of epithelium	Birds	(147, 148)
Fetal lung	Maturation, surfactant production	Mammals	(149–152)
Heart	↑ Ornithine decarboxylase (ODC) activity	Mammals	(153)
Liver	Hepatocyte proliferation	Mammals	(154)
	Activation via		
	Protein kinase C		(155, 156)
	Diacylglycerol		(156)
	MAP kinase		(157)
	Phosphoinositide turnover		(158)
	Induction of growth-related genes		
	IGF-1		(159)
	ODC	Reptiles, mammals	(153, 156, 160, 161)
	β-actin	Mammals	(156)
	c-*myc*		(156)
	c-*fos*		(162)
	c-*jun*		(162)
	c-*src*		(162)
	↓ Cytokine gene expression in Kupffer cells		(163)
	Induction of growth factors		
	Synlactin		(164–166)
	Liver lactogenic factor		(167)
Liver, kidney	DNA hypomethylation	Mammals	(168)
Kidney	Growth of tubular epithelium	Fish	(169, 170)
	↑ ODC activity	Mammals	(153)
Intestine	↑ Intestinal mucosa	Birds, mammals	(171–173)
	Growth and changes in metabolism		(174, 175)
Muscle	Proliferation of vascular smooth	Mammals	(176, 177)
	Induction of growth-related genes		
	c-*myc*		(178)
	ODC		(178)
Adipocytes	Preadipocyte differentiation	Mammals	(179)
Pancreas	Proliferation of β-cells	Mammals	(180–183)
	Increased β-cell-to-cell communication		(184–186)
Adrenal	↑ ODC activity	Mammals	(153)
Pituitary	Proliferation of GH3 cells (somatolactotrophs)	Mammals	(187)
Germ cells	↑ Maturation	Mammals	(188, 189)
Gonads	↓ Weight	Birds	(190)
	↑ Weight	Mammals	(191)
	↑ ODC activity		(192)

TABLE 3. Continued

Organ or target	Effect	Animal class	Reference
Prostate	Proliferation of human BPH epithelial cells	Mammals	(193)
	↑ Growth		(194)
	↑ ODC activity		(195)
Seminal vesicle	↑ Growth	Mammals	(196)
Amnion	↑ DNA synthesis and creatine kinase activity	Mammals	(197)
Brain	Astrocyte proliferation	Mammals	(198)
	Tuberoinfundibular hypothalamic development		(199)
Retina	Metamorphosis of visual pigments	Amphibians	(200)
Immune system	↑ Thymus and spleen weights	Mammals	(201)
	Proliferation of lymphocytes		(202, 203)
	Tumor growth		
Intestine	Colo-rectal tumor agessivity	Mammals	(204, 205)
Mammary	Proliferation of	Mammals	
	MXT cells		(206)
	MCF-7 cells		(207)
	T-47D cells		(208)
Lymphoma	Proliferation of malignant B lymphocytes (requires Ig-bound PRL)	Mammals	(209)
	Proliferation due to provirus insertion into PRLR promoter		(210)
	Proliferation of pre-T Nb2 cells		(211)
Promyelocytes	Proliferation of HL60 cell line	Mammals	(212)
Leiomyoma	Associated with ↑ PRL	Mammals	(213)

section of this review, there were some aspects that did not fit well with either the discussion on reproduction or immune function. In liver, in addition to the large number of activities that have already been reported, PRL is also able to increase free intracellular Ca^{2+} concentrations (223), $PGF_{2\alpha}$ and PGE production (232), and IGF-I production, at least under some circumstances (233).

Finally, many of the multiple functions of PRL in the numerous target tissues that have been identified (Table 1) are enhanced by an up-regulation of PRLRs induced by PRL itself. Thus, PRL is able to sensitize the response of certain tissues by increasing the number of specific PRLRs, representing the first step in the signal transduction process of the hormone (35).

D. Brain and behavior

1. Parental behavior. As shown in Table 5, PRL has been suggested to be involved in parental behavior of fishes, birds, and mammals. In fishes, PRL has been implicated in fin fanning, to provide a constant supply of fresh water to the eggs and to stimulate mucus production, which is used to feed the young after they hatch (108, 236). Another form of parental behavior is foam nest building, in which air bubbles are mixed with mucus to form bubbles during egg laying (237). PRL is also involved in migration. This hormone induces some forms of teleosts to migrate from seawater to fresh water (238) and causes certain forms of salamanders to actively seek and migrate to an aquatic habitat (109). PRL predisposes some species of birds to migrate, as is evidenced by increased nocturnal restlessness and food consumption (239). Birds also respond to PRL by an increase in nesting behavior, nest attendance, and incubation behavior (242–244).

Almost all adult female mammals show some form of maternal care toward their young offspring immediately after parturition, whereas the response of nulliparous females is less intense or completely absent. The endocrine changes that occur during and at the end of pregnancy, including increased circulating concentrations of PLs and PRL are thought to be responsible for the induction of maternal behavior (245–247). PRL is also considered to be responsible for inducing excessive grooming in rats (248–250). Increased food intake (hyperphagia) can be observed in birds and mammals in response to PRL (190, 241, 251, 252), and in birds, regurgitation feeding has also been shown to be PRL-induced (241, 254).

2. Others. Adaptive stress responses in mammals represent another set of behavioral responses that are induced by PRL (250, 255). Interestingly, PRL has been shown to have analgesic effects (256, 257) that can be mimicked by a number of central nervous system neurotransmitters, including opioids, γ-aminobutyric acid, acetylcholine, and Ca^{2+} and K^+ channels (258–262). In women, elevated levels of PRL are associated with some psychosomatic reactions, including a form of pseudopregnancy (263). In rats, PRL has been shown to induce increased lordosis behavior (270). Interestingly, the effect does not appear to be associated with rapid electrophysiological action but rather with a neuromodulatory or metabolic effect, since the latency is about 40 min. Elevated circulating PRL is also thought to be responsible for decreased libido (263, 264), increased rapid eye movement sleep (265–267), and an alteration of the sleep-wake cycle (268, 269).

In the hypothalamus, PRL has been suggested to be important for maturation of the neonatal neuroendocrine system (199, 275). In species such as rodents in which the young are born immature, PRL may be provided by maternal milk, which can be absorbed by the intestinal epithelium of neonatal animals for a few days. As much as 20% of ingested PRL is thought to reach the fetal circulation (283). PRL is also

TABLE 4. Endocrinology and metabolism

Organ	Effect	Animal class	Reference
	Energy Metabolism		
Brain	Tissue-specific modulation of ATPases	Mammals	(216)
	Lipid Metabolism		
Fetal lung	↑ Phospholipid synthesis	Mammals	(149)
Liver	↑ Lipoprotein lipase activity	Mammals	(217)
	↑ Bile secretion		(218)
	↑ Na^+/taurocholate co-transport		(219, 220)
Adipocyte	↑ Lipoprotein lipase activity	Birds	(221)
	Carbohydrate Metabolism		
Brain	Modification of enzyme activities	Mammals	(222)
Liver	↑ Glycogen phosphorylase a activity	Mammals	(223)
Pancreas	↑ Insulin secretion	Mammals	(185, 224, 225)
	↓ Glucose threshold for insulin secretion		(226)
	↑ Glucokinase and glucose transporter 2		(227)
	Steroid Metabolism		
Adrenal	↑ Steroidogenesis	Mammals	(228, 229)
	↑ Adrenal androgens (DHEA, DHEA-S)		(229)
	↑ Cortisol, altosterone		(228)
	↑ 21-Hydroxylase activity		(230)
Skin	↑ Steroidogenic enzyme (3βHSD)	Mammals	(231)
	Signal Transduction		
Liver	↑ Ca^{2+} concentration	Mammals	(223)
	↑ $PGF_{2\alpha}$ and PGE		(232)
	↑ IGF-I production		(233)
Many tissues	↑ PRLR number		(35)

DHEA, Dihydroepiandrosterone; DHEA-S, dihydroepiandrosterone sulfate.

known to increase the turnover of dopamine (271–274), to increase the electrical activity of ventral hypothalamic neurons (279), and to augment PKC activity (280). Finally, two effects of PRL have been observed in the retina, the first a decline in the level of TRH receptors (281), and the second, an increase in photoreceptor destruction (282).

E. Reproduction

Actions related to the processes of reproduction represent the largest group of different functions that have been identified for PRL. These are listed in Table 6. The different actions are quite diversified, but certain subcategories can be identified.

1. Nurturing of young. PRL is best known for its actions on the mammary gland. In this complex organ, the growth of the gland that occurs during pregnancy is under the control of a number of trophic factors including estrogen, progesterone, insulin, glucocorticoid, GH, and PRL or PL. The terminal stage of mammary gland development, lobuloalveolar growth, is directly regulated by PRL (93). Although the hormonal requirements for the induction and maintenance of milk production vary in different species, the common factor is that PRL is the hormone primarily responsible for the synthesis of milk proteins (284), lactose (290), and lipids (292), all major components of milk. In addition, PRL has been shown to directly stimulate IGF-I binding protein (296), epidermal growth factor (EGF) (297), a glycosylated mucin (299), parathyroid-like peptide (301), and PRL-inducible proteins (300) in normal and neoplastic mammary tissue.

Although birds do not have a mammary gland, the esophagus of some birds leads to a large chamber known as the crop sac, which connects to the avian stomach. The crop sac serves as an organ for food storage. In pigeons and doves, the crop sac has developed a high degree of sensitivity to PRL, as this hormone is able to stimulate growth of the crop sac, including proliferation and thickening of the epithelium. As the cells move away from the basal layer, they hypertrophy and accumulate fat globules. These cells are finally sloughed off into the crop lumen forming a substance known as crop milk, which is used to feed the young (241). Recently, a number of genes have been shown to be induced in the pigeon crop sac in response to PRL, including annexin Icp 35 (302, 303), lipoprotein lipase (221), ornithine decarboxylase (305), as well as other proteins of unknown function (304, 306).

As has been mentioned above, in some fish, modified skin glands are able to produce mucus, which serves as nourishment for the young. This mucous secretion in many fish is responsive to PRL (108). Thus, as is seen for birds and fish, the production of a substance for the survival of the young is not a feature restricted to mammals.

2. Ovarian actions. In rodents, luteotropic and luteolytic actions of PRL have been recognized for a number of years (307–309). Since progesterone production by the ovaries is necessary for implantation of the fertilized ovum, for maintenance of pregnancy, and for inhibition of ovulation, regulation of progesterone production by the corpus luteum is an important feature in reproduction. In general, the luteotropic action of PRL (338) involves stimulation of progesterone production by luteal cells (341). Progesterone synthesis

TABLE 5. Brain and behavior

Organ or target	Effect	Animal class	Reference
Brain, CNS	Fin fanning; provides fresh water to eggs	Fish	(236)
	Foam nest building		(237)
	Migration	Fish, birds	(238, 239)
	Migration-water drive	Amphibians	(109)
	Nesting behavior	Birds	(136, 240)
	Nest attendance		(136, 241)
	Incubation behavior		(242–244)
	Maternal behavior	Mammals	(245–247)
	Grooming behavior		(248–250)
	Feeding behavior	Birds	(251)
	Hyperphagia	Birds, mammals	(190, 241, 252, 253)
	Regurgitation feeding	Birds	(241, 254)
	Adaptive stress responses	Mammals	(250, 255)
	Induced analgesia mimicking		(256, 257)
	Opioids		(258)
	GABA		(259)
	Acetylcholine		(260)
	Ca^{2+} channels		(261)
	K^+ channels		(262)
	Psychosomatic reaction (pseudopregnancy)		(263)
	↓ Libido		(263, 264)
	↑ REM sleep		(265–267)
	Sleep-wake cycle		(268, 269)
	↑ Lordosis behavior		(270)
Hypothalamus, striatum	↑ Dopamine turnover	Mammals	(271–274)
Hypothalamus	Maturation of neonatal neuroendocrine system	Mammals	(199, 275)
	↓ GnRH secretion		(276, 277)
	↓ Frequency and pulsatility of LH		(278)
	↑ Electrical activity of the VMH neurons		(279)
	↑ PKC activity		(280)
Retina	↓ TRH receptors	Mammals	(281)
	↑ Photoreceptor destruction		(282)

VMH, Ventromedial hypothalamus; REM, rapid eye movement.

is, in turn, affected by activation and inhibition of ovarian steroidogenic enzymes (312–314, 322, 327, 341). Since the maintenance of ovarian progesterone production involves a luteotropic complex rather than a single gonadotropin, PRL certainly acts in concert with LH/hCG and perhaps other factors.

An antigonadal effect of PRL, involving a marked reduction in the weight of the gonads, has been observed in birds (190). In mammals, depending on the stage of the cycle, luteolytic effects of PRL have also been reported (339, 340). Several factors, including PRL, seem to be involved in the destruction of the corpus luteum. In granulosa cells, PRL inhibits estrogen synthesis (319–321) and P450 aromatase (322) and induces α_2-macroglobulin via activation of Stat5 (336). As a factor regulating the formation and destruction of the corpus luteum, PRL seems to play a major role in modulating the physiological states of estrus, pregnancy, and lactation.

A direct effect of PRL on developmental competence and maturation of oocytes has been reported in rabbits (188, 337). The addition of PRL to the oocyte maturation medium increased the development of organized embryos. In addition, PRL was able to directly inhibit degeneration and decomposition of surface epithelial cells and the disruption of connective tissue at the apex of the follicle wall. PRL inhibited the activity of hCG-stimulated plasminogen activator in mature follicles. These latter studies suggest that the preovulatory PRL environment can influence oocyte maturation.

3. Uterine actions. In the uterus, PRL is able to increase the level of progesterone receptors, and thus all actions associated with this steroid hormone are enhanced (344, 345). PRL has been reported to induce uterine fluid loss (348) and decrease progesterone metabolism (347). A stimulatory effect is also observed on estrogen receptor levels (349, 350), as well as in conjunction with estrogen, on the general secretory activity of the endometrium (344, 352). PRL promotes blastocyst implantation (355) and increases uteroglobin production (354), leucine aminopeptidase activity (353), and glucose amine synthetase activity (355). Finally, stimulation of prostaglandins and phospholipase A_2 is observed (346).

4. Testicular actions. The physiological role of PRL in males has puzzled investigators ever since the hormone responsible for mammary gland development and lactation in females was shown to be present in the anterior pituitary of males. Initially, no clear function could be ascribed to PRL in male animals or humans (93). More recent data have shown, however, that in general, PRL stimulates testicular functions in most mammals, although as has been reported previously, in birds it causes a decrease in the weight of the gonads (190). In Leydig cells, PRL is involved in the maintenance of cellular morphology (189), increases LH receptor number (191, 356),

TABLE 6. Reproduction

Organ or target	Effect	Animal class	Reference
Mammary gland	Lobuloalveolar growth	Mammals	(93)
	↑ Milk protein synthesis		(284)
	α-Casein		(285)
	β-Casein		(286)
	Whey acidic protein		(287)
	β-Lactoglobulin		(288)
	Late lactation protein of marsupials		(289)
	↑ Lactose synthesis		(290)
	Lactose synthetase		(291)
	Galactosyl transferase		(291)
	α-Lactalbumin		(291)
	↑ Lipid metabolism		(292)
	Acetyl-CoA carboxylase		(293)
	Fatty acid synthase		(294, 295)
	Malic enzyme		(294, 295)
	Lipoprotein lipase		(294, 295)
	↑ IGF-I binding protein		(296)
	↑ EGF		(297)
	↑ 120-kDa protein		(298)
	↑ Muc 1 (glycosylated mucin)		(299)
	↑ PRL-inducible protein		(300)
	↑ Parathyroid-like peptide		(301)
Crop sac	Growth	Birds	(241)
	↑ Mitosis of germinal layer		(147)
	Thickening of epithelium		(147)
	↑ Annexin Icp 35		(302, 303)
	↑ Crop milk polypeptide 58 and 50.5		(304)
	↑ Lipoprotein lipase		(221)
	↑ Ornithine decarboxylase		(305)
	↑ 25-kDa protein		(306)
Ovary	Luteotropic and luteolytic actions (dependent on stage of estrous cycle)	Mammals	(307–309)
	Ovum maturation		(310)
	↓ Folliculogenesis		(311)
	↓ 3β-HSD		(312)
	↓ Aromatase		(313)
	Potentiate effects of LH on 3β-HSD		(314)
	↓ Ovulation		(315)
	↓ Estradiol and induce ovarian regression	Birds	(316)
	↓ Plasmin generation in preovulatory follicles	Mammals	(317, 318)
Granulosa cells	↓ Estrogen production	Mammals	(319–321)
	↓ P450 aromatase		(322)
	↑ Use of extracellular lipoproteins		(323, 324)
	↑ Progesterone production		(320, 324, 325)
	↑ LH receptors		(326)
	↑ 20α-OH progesterone (humans)		(327)
	↓ Progestins (humans)		(328–330)
	↓ Luteinization		(331)
	↓ cAMP and steroidogenesis (rats)		(332)
	Counteract morphological effects of LH		(333)
	↑ Progesterone in cocultures of splenic macrophages removed at proestrus		(334)
	↑ DAG		(335)
	↑ 101_2-Macroglobulin		(336)
Oocytes	↑ Developmental competence and maturation (rabbits)	Mammals	(188, 337)
Luteal cells	Luteotropic action	Mammals	(338)
	Luteolysis in pregnancy (marsupials)		(339)
	Luteolysis during estrous cycle		(340)
	↓ 20α-HSD		(341)
	↑ Progesterone		(307)
	Control of delayed implantation and steroidogenesis (bats)		(342)
	↓ 37-kDa protein		(343)

TABLE 6. Continued

Organ or target	Effect	Animal class	Reference
Uterus	↑ Progesterone receptors and progesterone effects	Mammals	(344, 345)
	↑ PGE_2, phospholipase A_2, prostaglandin G/H synthase		(346)
	↑ Fluid loss		(347)
	↓ Progesterone metabolism		(348)
	↑ Estrogen receptors		(349, 350)
	↓ Myometrial contraction		(351)
	↑ General secretory activity of endometrium (with estrogen)		(344, 352)
	↑ Leucine aminopeptidase activity (with estrogen)		(353)
	↑ Uteroglobin production		(354)
	Promote blastocyst implantation		(355)
	↑ Glucose amine synthetase activity		(355)
Testis	In general, ↑ activities	Mammals	
Leydig cells	Involved in maintenance of cell morphology	Mammals	(189)
	↑ LH receptors		(191, 356)
	↓ Aromatase activity (with LH)		(357)
	↑ Steroidogenesis and androgen production (cooperation with LH)		(356, 358–360)
			(191)
Sertoli cells	↑ FSH receptors	Mammals	(361)
Germ cells	↑ Total lipids	Mammals	(362)
	↑ Spermatocyte-spermatid conversion		(189)
Spermatozoa	↑ Ca^{2+} binding and/or transport of ejaculated and epididymal spermatozoa	Mammals	(363)
	Energy metabolism:		(364)
	↑ ATPase activity		(365)
	↑ Fructose rate		(366)
	↑ Glucose oxidation		(364, 367)
	↓ Zn^{2+} content		(368)
	Maintenance of mobility and attachment to oocyte		(369)
	Shortening optimal preincubation period to acquire capacitation		(369)
Epididymis	Energy metabolism:	Mammals	
	↑ Glycogenolysis and hexophosphate enzymes		(370)
	↑ Sialic acid		(371)
	↑ β-galactosidase and α-mannosidase activities		(372)
	↑ Lipids		(373)
	↓ Glycoprotein metabolism		(374)
Seminal vesicle	Hyperprolactinemia: ↓ sperm fertilizing and mobility capacity	Mammals	(375, 376)
	Hypoprolactinemia: azoospermia		(377)
	↑ Fluid lipids		(378)
	↑ Lipogenesis		(379)
	↑ Phosphomonoesterase and acid phosphatase		(380)
	↓ Glycosylation		(381)
Prostate	↑ Weight	Mammals	(123)
	↑ Nuclear uptake of DHT		(382–384)
	↑ Androgen receptor		(385, 386)
	Involvement in estrogen-induced inflammation		(387)
	↑ Epithelial secretory function		(381, 388)
	↑ Energy metabolism:		
	Monosaccharide formation		(389)
	Amino acid oxidation and transamination		(390)
	↑ Ornithine decarboxylase		(195)
	↑ Citric acid secretion:		(391)
	↑ Mitochondrial aspartate		(392)
	Aminotransferase (via PKC)		
	Citrate oxidation and m-asconitase:		(393)
	Ventral prostate: ↑		
	Dorsal prostate: ↓		
	↑ Pyruvate dehydrogenase E1α		(394)
	↑ Aspartate transporter		(391)
	↑ C3 subunit of prostatein, probasin, RWB gene		(395)
	↑ IGF-1 and IGF-1 receptor		(386)

HSD, Hydroxysteroid dehydrogenase; DAG, diacylglycerol; DHT, dihydrotestosterone.

TABLE 7. Immunoregulation and protection

Organ or target	Effect	Animal class	Reference
Spleen	↑ Weight	Mammals	(201)
Thymus	↑ Weight	Mammals	(201)
	↑ Thymulin production		(397)
Submandibular gland	↑ Immunostimulatory activity	Mammals	(398)
Lymphocytes	↑ Hormonal immunity	Mammals	(399)
	↑ Adjuvant arthritis response		(399–401)
	↑ Cellular immunity		(402)
	↑ Antibody formation to sheep RBC		(403)
	↑ IgG and IgM antibodies		(404–406)
	Reverse hypophysectomy-induced anemia, leukopenia, and thrombocytopenia		(407)
	↑ Proliferation		(203, 408–410)
	↑ IL-2 receptors		(203, 411)
	↑ EPO receptors		(412)
	↑ PRL receptors		(413, 414)
	↓ Apoptosis		(415, 416)
	↑ IFN-γ		(417)
	↑ Graft rejection		(418)
	↑ c-*myc*		(201)
	↑ DNA synthesis		(201)
	↑ T cell engraftment		(419)
	↑ DNA synthesis	Birds	(420)
Nb2 cells	↑ Proliferation	Mammals	(211)
	↓ Apoptosis		(416, 421)
	Genes induced:		
	IRF-1		(422, 423)
	c-*myc*		(424)
	c-*fos*		(424)
	Ornithine decarboxylase (ODC)		(425)
	hsp 70		(426)
	β-Actin		(427)
	pim-1		(428)
	gfi-1		(429)
	bcl-2		(430)
	bax		(430)
	T-cell receptor γ-chain		(431)
	Cyclins D2 and D3		(432)
	Cyclin E, *cdk2*, *cdk5*, E2F-1		(433)
	Clone 15, nuclear movement protein		(434)
	GnRH		(435)
	GnRH receptor		(435)
	Proteins activated:		
	Jak2		(436–439)
	Fyn		(440)
	Stat proteins		(436, 441, 442)
	Ras		(443, 444)
	Raf		(445)
	Vav		(446)
	Grb2		(444)
	Sos		(444)
	Shc		(444)
	MAP kinase		(443, 447)
	Stathmin		(448, 449)
	IRS-1		(450)
	PTP-1D (SHP-2)		(451)
	PKC		(452, 453)
	Casein kinase II		(454)
	PTK		(452)
	PI3 kinase		(450, 455)
	cbl		(456)
	S6 kinase		(457)
	G proteins		(458, 459)
	PLC		(460)
	Amiloride-sensitive Na^+/H^+ exchange system		(461)
NK cells	↑ Susceptibility of primary leukemia cells	Mammals	(462)
	↑ Cytotoxic effects		(463)
	↑ DNA synthesis		(464)

TABLE 7. Continued

Organ or target	Effect	Animal class	Reference
Macrophages	↑ Activation	Mammals	(408)
	↑ Cytokine gene expression in Kupffer cells following hemorrhage		(163)
	↓ Monoblastic growth-synergy with IFN-γ		(465)
	↑ Superoxide anion responsible for killing pathogenic organisms		(466, 467)
	↑ Nitric oxide and protection against bacterial infection		(468)
Polymorphonuclear cells	↓ Direct and spontaneous migration	Mammals	(469)
Thymic nurse cell complexes	Regulation of lymphocyte/epithelial cell adhesive interactions	Mammals	(470)
Mammary gland	↑ IgA-secreting plasma cells	Mammals	(471)
Liver	Induction of coagulation factor XII	Mammals	(472)
Skin	Melanogenesis	Fish	(142)
	Pathology		
Lymphocytes	Treatment for immunodepression following severe hemorrhage	Mammals	(473)
	↑ Autoimmune responses		
	Systemic lupus erythematosus		(405, 474, 475)
	Acute experimental allergic encephalomyelitis		(476)
	Rheumatoid arthritis		(477)
	Adjuvant arthritis		(399, 400, 478)
	Graft *vs.* host disease		(479)

and along with LH, decreases aromatase activity (357) and increases steroidogenesis and androgen production (356, 359, 360). In Sertoli cells, PRL has been shown to increase FSH receptor numbers (361). In germ cells, PRL increases total lipids (362) and increases the spermatocyte-spermid conversion (189). Several effects on spermatozoa have been reported, including an increase of calcium binding and/or transport of ejaculated and epidymal spermatozoa (363) as well as an increase in energy metabolism (364), a maintenance of mobility and attachment to the oocyte (369), and a reduction in the time required to achieve capacitation (369).

5. Male sex accessory actions. In rats, effects on accessory sex organs are well established. In addition to increasing the weight of the prostate and seminal vesicle, in conjunction with androgens (194, 196), PRL also has metabolic effects on sex accessory organs. In the epididymis, energy metabolism is increased (370) and, in addition, sialic acid and lipid levels are augmented (371, 373). In the seminal vesicle, PRL increases lipid concentrations in the fluid (378), lipogenesis (379), and phosphomonoesterase and acid phosphatase activities (381). The effects of PRL on prostate include increased levels of androgen receptor (385, 386), involvement in estrogen-induced inflammation (387), increased epithelial secretory function (381, 388), augmented energy metabolism, and an enhancement of citric acid production (391). PRL has also been reported to stimulate the level of IGF-I and IGF-I receptor in the prostate (386) and to increase the production of other prostate-specific proteins (395).

F. Immunoregulation and protection

Although actions of PRL on the hematopoietic system were suggested in early studies involving treatment of hypophysectomized rats with PRL (396), it was not until much later that a clear role of PRL in the immune system was established. Table 7 summarizes the multiple effects of PRL in the regulation of immune function. Injection of PRL into hypophysectomized rats causes an increase in the weight of the spleen and thymus (201). In addition, PRL activates an immunostimulatory action of the submandibular gland (398) and augments the production of a thymic hormone, thymulin (397).

In lymphocytes, PRL is known to increase hormonal and cellular immunity (399, 402), to reverse anemia, leukopenia, and thrombocytopenia induced by hypophysectomy (407), to increase antibody formation, including IgG and IgM antibodies (404–406), and to induce cellular proliferation (203, 408–410), although this last function is somewhat controversial, as not all investigators are able to reproduce PRL-induced proliferation of lymphocytes. Recently, in fact, it has been suggested that the proliferative action of PRL of recombinant origin and even natural preparations may be due to contamination with endotoxins, which are responsible for the immunostimulatory actions that have been observed (480). This interesting observation awaits confirmation. Along with effects on proliferation, PRL has also been shown to increase DNA synthesis, and *c-myc* expression (201).

PRL has also been reported to increase receptor levels for interleukin (IL)-2 (203, 411), EPO (412), and PRL (413, 414). Recently, in addition to stimulating proliferation, PRL has been shown to inhibit apoptosis of lymphocytes (415, 416)(see below). Administration of PRL is also associated with increased graft rejection (418, 481) and an increase in T cell engraftment (419). In natural killer cells, PRL has been reported to increase DNA synthesis (464) and augment cytotoxic effects (463), as well as increasing susceptibility of primary leukemic cells (462).

Activation of macrophages was originally thought to be an action of GH. More recently, however, it has been shown that macrophage activation (408) and superoxide anion production responsible for killing pathogenic organisms (466, 467) are effects mediated by the PRLR. The protective effect against *Salmonella* infection appears to be mediated by nitric oxide production (468). In synergy with IFN-γ, PRL decreases monoblastic growth (163) and increases cytokine gene expression in Kupffer cells after hemorrhage (163).

PRL reduces direct and spontaneous migration (469) of polymorphonuclear cells (469), regulates lymphocyte-epithelial cell adhesive interactions in the thymic nurse cell complex (470), and augments IgA-secreting plasma cells in mammary gland (471).

In terms of more specific protective effects, PRL induces the production of coagulation factor XII by the liver (472) and, in fish, regulates melanogenesis (144).

Finally, as listed in Table 7, multiple functions of PRL are observed in the pre-T lymphoma Nb2 (for review, see Ref. 482). These cells respond to very low concentrations of PRL by a marked proliferation (211) and, in fact, are used routinely as a bioassay of PRL (483). Within the past few years, studies on Nb2 cells have also highlighted the antiapoptotic properties of PRL on lymphoid cells. This effect appears mediated by gene products known to be involved in apoptosis, such as Bcl-2 and Bax (416, 430), although involvement of newly identified proteins such as *pim*-1 (428) or *Bag*-1 (484) has also been proposed. Almost all the activities reported for Nb2 cells in Table 7 are associated with the mechanism of action of PRL: these include the induction of many growth-related genes, and the activation of a number of proteins implicated in the signal transduction process. These effects are discussed in detail in *Section VI.*

G. Actions associated with pathological disease states

In humans, hyperprolactinemia has been shown to be associated with amenorrhea, galactorrhea, and impotence (485). The inhibitory effects on the reproductive processes may be due to both central and peripheral actions of PRL. In some women, elevated PRL is associated with a psychosomatic state of pseudopregnancy (263, 264).

Interestingly, however, no genetic diseases associated with a mutation of the gene encoding PRL or the PRLR have been identified in humans or animals. Either these genes are not important or they are essential to the proper survival of the species. As described below, knockout of the PRLR gene in mice is not lethal but does produce major reproductive defects in females, which would, of course, affect reproductive function and survival.

An excessive volume of amniotic fluid, known as polyhydramnios, is associated with decreased levels of amniotic fluid PRL (120) or PRLR levels in the chorion laeve (486). This effect may be related to the osmoregulatory role of PRL during fetal life and to the inhibitory effect on amniotic fluid volume observed in monkeys (119).

PRL has been associated with a number of different forms of cancer. For example, PRL is thought to increase colorectal tumor agressivity (204, 205), induce the proliferation of several lines of human breast cancer (206–208), activate malignant B lymphocytes (209) and lymphoma cells (211), and induce the proliferation of promyelocytes (212). Benign fibromuscular myometrial tumors (leiomyomas) have been shown to produce more PRL than control myometrium (213); thus, locally produced PRL may exert a mitogenic action on the growth of these tumors.

PRL has been shown to be increased and to effect a number of autoimmune states, such as systemic lupus erythematosus (405, 474, 475), acute experimental allergic encephalomyelitis (476), rheumatoid arthritis (477), adjuvant arthritis (478), and graft *vs.* host disease (479), *e.g.*, as a marker of rejection in heart transplantation (481). PRL has also been suggested to be involved in the etiology of cystic fibrosis (121), although the precise mechanism remains unclear.

VI. Signal Transduction by the PRLR: Structure-Function Relationships

All of the actions described above result from the interaction of PRL with its receptor in various target cells, which leads to the activation of a cascade of intracellular events. In this section, we summarize the current state of knowledge concerning signal transduction of the PRLR. As indicated previously, Nb2 cells are one of the preferred models with which to study PRL actions, and a list of genes induced and proteins activated by lactogenic stimulation of these cells can be found in Table 7.

A. The JAK-Stat pathway

1. Janus kinases.

JAK family. The cytoplasmic tail of the PRLR, whatever the isoform, is devoid of any consensus sequence for enzymatic activity, including kinase activity (35, 487), as are all cytokine receptors identified thus far. However, hormonal stimulation of the PRLR leads to tyrosine phosphorylation of several cellular proteins, including the receptor itself (488), and tyrosine kinase inhibitors were shown to inhibit the mitogenic and anabolic effects of lactogenic hormones in lymphoid Nb2 cells (489). Until 4 yr ago, this observation was unsolved. In the early 1990's, Wilks and colleagues (490, 491) identified, by low-stringency hybridization and PCR approaches, a new family of protein tyrosine kinases to which they gave the acronym JAK (for just another kinase). Regarding the presence of two kinase-like domains in these kinases, the name JAK was also proposed as an acronym for Janus kinase, in reference to the dual-faced Roman god Janus (492). To date, the JAK family includes four members, termed JAK1, JAK2, JAK3, and Tyk2 (reviewed in Ref. 493). All cytokine receptors work in combination with one or several JAKs to transmit the hormonal signal within the cell (41, 44, 45, 493–495). In 1994, as demonstrated 1 yr before for the GHR (496), JAK2 was identified by three laboratories as the JAK kinase associated with the PRLR (438, 439, 497). Since this major discovery, important steps in understanding the complex field of signal transduction by the PRLR have been made and are summarized in the following sections. A schematic representation of the current knowledge of the main PRLR-activated signaling pathways is proposed in Fig. 3.

Association of JAK2 with the PRLR. Although involvement of JAK1 has also been proposed in the particular context of mouse lymphoid BAF/3 cells transfected with the PRLR (498), JAK2 is unambiguously the major PRLR-associated Janus kinase. Using mutant cell lines defective in JAK1 or JAK2, Stark and colleagues (499) have very recently confirmed this assumption and showed that although milk protein gene induction required the latter, the former was dispensable. Possible interactions between JAK2 and JAK3 in avian PRLR signaling have been suggested, but await further

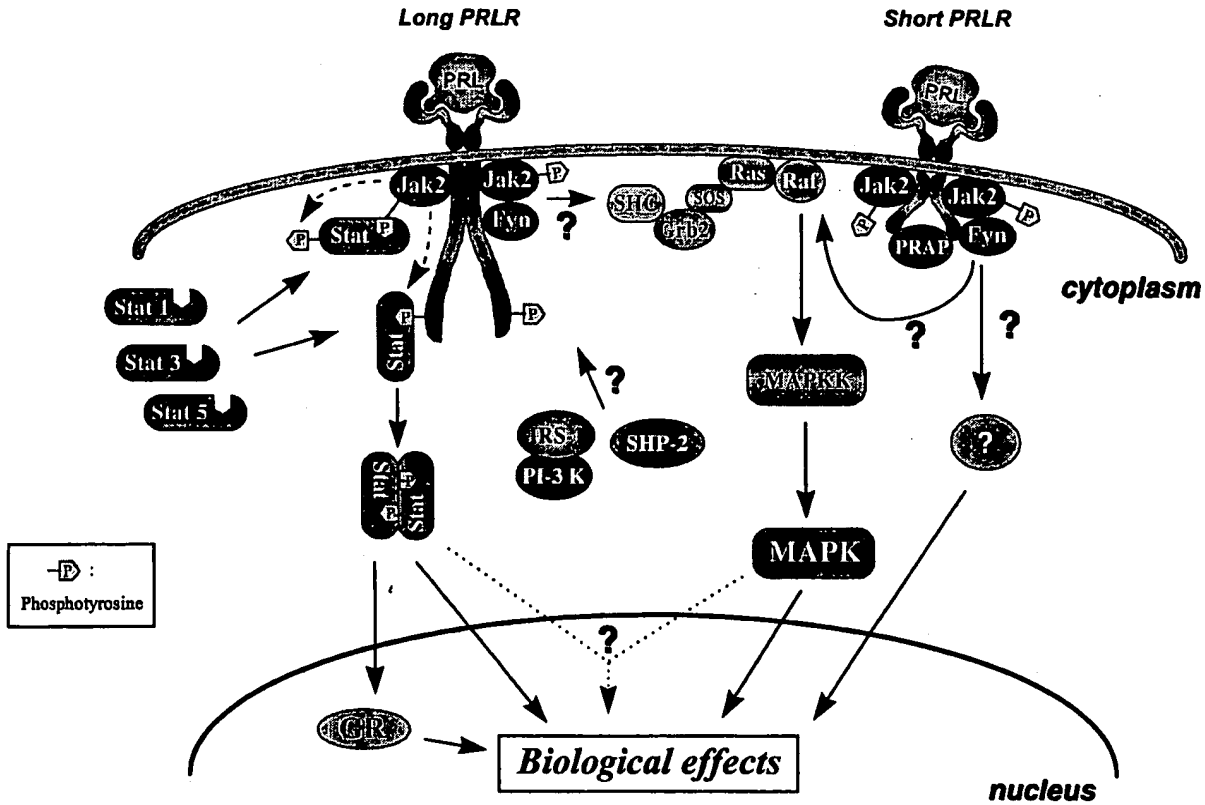

FIG. 3. Schematic representation of the PRLR signaling pathways. Long and short isoforms of rat PRLR are represented. PRLR activates Stat1, Stat3, and, mainly, Stat5. Interaction of Stat5 with the glucocorticoid receptor (GR) has been reported. Whether the short PRLR isoform activates the Stat pathway is currently unknown. PRAP (PRLR-associated protein) seems to interact preferentially with the short PRLR. The MAP kinase pathway involves the Shc, Grb2, Sos, Ras, Raf cascade and is presumably activated by both PRLR isoforms. Connections between the JAK-Stat and MAPK pathways have been suggested. Interactions between receptors and Src kinases (*e.g.*, Fyn), SHP2, IRS-1, PI-3 kinase, and other transducing molecules remain unclear.

confirmation (59). JAK2 is constitutively associated with the PRLR (439, 500), *i.e.*, it is not induced by ligand binding, contrary to what has been observed for the GHR (496). The PRLR-JAK2 interaction involves the membrane-proximal region of the PRLR cytoplasmic domain, in agreement with the ability of the short PRLR isoform to associate with the kinase (501). This region includes the consensus box 1, which is highly enriched in proline residues (I^{243}-P^{244}-P^{245}-P^{246}-V^{247}-P^{248}-G^{249}-P^{250} in rat PRLR) and is believed to adopt the typical folding of SH3-binding domains (502). Alanine substitutions of individual residues within box 1 of the rat PRLR have shown that the most C-terminal proline (P^{250}) is critical for association with and subsequent activation of JAK2 (503). However, sequence analysis of Janus kinases failed to identify any consensus SH3 motif. Current knowledge thus suggests either that an adapter links JAK2 and the PRLR, by interacting with the PRLR box 1 through an SH3 domain and with JAK2 through another mechanism, or that the receptor-kinase interaction involves a mechanism different from the known SH3-SH3 binding domain. In agreement with the latter hypothesis, O'Neal and colleagues (504) recently proposed that peptides including the amino acid sequence of PRLR box 1 adopt a folding different from that of classic SH3-binding domains and, therefore, are not able to interact with protein SH3 domains. These authors also suggested that isomerization of the second proline of the consensus P-x-P motif might regulate the activation of the receptor in a "on/off" switch manner (504). Although this proposal relies on observations made in the particular context of spectrometric analysis (circular dichroism, nuclear magnetic resonance) of peptides, it is in good agreement with previously reported mutational studies emphasizing the functional importance of the second proline residue of the P-x-P motif in both the PRLR and the GHR (503, 505–507). Finally, although these data clearly demonstrate that the membrane-proximal box 1 is an absolute requirement for associating with the kinase JAK2, involvement of additional residues toward the C terminus cannot be ruled out. In this respect, that the wild-type rat short PRLR (291 aa) associates with JAK2 in fibroblasts transfected with a plasmid encoding this receptor (501) whereas a mutant form of the long PRLR truncated 11 residues upstream (analog named T280, containing the 280 N-terminal aa) does not (437, 508), might reflect some subtle involvement of the C-terminal tail of the short PRLR in JAK2 binding (see below).

To the best of our knowledge, no mutational study aimed at mapping the region of JAK2 interacting with the PRLR has yet been reported. However, it was recently shown that the N-terminal fifth of JAK2 is involved in GHR binding (509); whether this region binds directly to the GHR is unknown.

Activation of JAK2 by the PRLR. It is usually assumed that activation of Janus kinases occurs by transphosphorylation of tyrosines upon ligand-induced oligomerization of cytokine receptors, which brings two JAK molecules close to each other (44, 495). Based on JAK kinase activation by chimeric receptors in which various ECDs of cytokine or tyrosine kinase receptors were fused to the IL-2 receptor β-chain, we have recently suggested that the stoichiometry of oligomerized cytokine receptors, namely homodimerization, might be involved in the specific recruitment of JAK2 by the PRLR (509a). Identification of tyrosine residues of JAK2 that are phosphorylated after PRLR activation remains to be performed. As expected, PRLR mutants unable to associate with JAK2, such as a box 1-deleted PRLR (501) or the C-terminal-truncated form T280 (437), are also unable to induce tyrosine phosphorylation of the kinase. An elegant experiment using chimeras of GM-CSF/PRL receptors (extracellular of GM-CSFR α and β, intracellular of PRLR) recently showed that heterodimerization of the short and the intermediate PRLR cytoplasmic tails achieves inactive complexes unable to stimulate JAK2 autophosphorylation (510), whereas both have been reported to associate with and activate the kinase in the context of their respective wild-type receptor (501). These data, suggesting that two copies of box 1 alone are insufficient for achieving JAK2 activation (510), are in agreement with results obtained with the T280 truncated long PRLR (437) but contradict the ability of short PRLR to activate JAK2 (501). These discrepancies may result from structural disturbance in the particular context of modified receptors (deleted or chimeric), but could also emphasize the functional significance of the 30 C-terminal residues of the short rat PRLR (aa 262–291; Fig. 2A), which differ from the equivalent region in the intermediate and long PRLR isoforms (35) and, thereby, might confer distinct properties to the receptor whether homodimerization or heterodimerization occurs. Based on further analysis of GM-CSFR/PRLR chimeras, Clevenger and colleagues (510a) recently showed that heterodimerization of the wild-type cytoplasmic domain of the intermediate PRLR with any truncated form of this PRLR, even including box 1, was detrimental to JAK2 autophosphorylation. They also observed that mutation of both tyrosines 309 and 382 within a single PRLR chain prevents JAK2 activation, which strengthens the belief that juxtaposition of strictly identical cytoplasmic domains is required for proper activation of the kinase. In this respect, we have recently shown, using several cell lines [Chinese hamster ovary (CHO) cells, 293 human fibroblasts, bovine mammary gland epithelial (BMGE) cells], that the short PRLR functions as a dominant negative isoform, inhibiting the activation of milk protein gene transcription by the receptor complex through heterodimerization. Indeed, in human embryonic kidney 293 fibroblasts cotransfected with cDNAs encoding the short and long PRLR isoforms, such heterodimers are shown to occur and result in an absence of JAK2 activation (511, 512). All these observations are in good agreement with our proposal that the homodimeric stroichiometry of some cytokine receptors is one of the major features leading to activation of JAK2 (509a).

Activation of JAK2 by the PRLR occurs very rapidly after hormonal stimulation (within 1 min, see Ref. 501). This suggests that the Janus kinase occupies a central and very upstream role in the activation of several signaling pathways of the PRLR. Accordingly, PRLR mutants that fail to associate with and/or to activate JAK2 were all reported inactive in any of the bioassays performed (437, 499, 501, 503, 510). When activated, JAK2 phosphorylates tyrosine residues on different target proteins, the best identified of which are the receptor itself (next paragraph) and a family of transducing proteins termed Stat (signal transducer and activator of transcription) (see *Section VI.A3*).

2. *PRLR phosphorylation.* Although the three PRLR isoforms are able to activate JAK2 (see above), the short isoform does not undergo tyrosine phosphorylation (501) in spite of the presence of four tyrosines in its cytoplasmic domain (Fig. 2A). Since phosphotyrosines are potential binding sites for transducer molecules containing SH2 (or any other phosphotyrosine recognizing) domains, particular attention is usually devoted to these amino acids, which play a central role in receptor signaling. In the intermediate PRLR isoform, which contains only three tyrosine residues in its cytoplasmic domain, we have identified the most C-terminal (Tyr 382) as the sole tyrosine undergoing phosphorylation upon receptor/JAK2 activation (513). Substitution of the equivalent tyrosine (Tyr 580) for a phenylalanine in the long isoform does not entirely abolish receptor tyrosine phosphorylation, however, indicative of the presence of at least one other potential phosphorylation site among the eight remaining tyrosines (513). To determine these potential phosphorylation sites, we recently performed a selected mutagenesis of each tyrosine of the rat long PRLR (514). Our results indicate that, in addition to Tyr 580, PRL is able to stimulate phosphorylation of tyrosines 473 and 479 in the basal state, but that in cells overexpressing JAK2, tyrosines 309, 402, and 515 can be phosphorylated in the particular context of PRLR containing a single tyrosine residue (Fig. 2B). These data can be correlated with those obtained from similar studies performed on the GHR, which is homologous to the long PRLR and also contains several tyrosine residues (nine for the rabbit GHR, eight for porcine GHR). Using point-mutated, domain-deleted, or C-terminal-truncated GHR analogs, several tyrosine residues have been mapped as potential phosphorylation sites (515–521). Taken together, these experiments performed on two closely related receptors indicate that tyrosine residues able to undergo phosphorylation are numerous and may be redundant, as illustrated by the intermediate PRLR isoform, which contains a single phosphorylation site and, so far, has been shown functionally equivalent to the long PRLR isoform (513, 522). To the best of our knowledge, no correlation between tyrosines that are preferentially phosphorylated by JAK kinases and their surrounding amino acids has been identified.

Despite the potential phosphorylation of several tyrosine residues within the long PRLR, the most C-terminal (Tyr 580) appears critical for PRLR functional activity since a PRLR mutant containing this sole tyrosine can mimic the wild-type receptor in stimulating reporter genes containing PRL-inducible sequences such as the β-casein promoter or Stat5 DNA-binding sites, while any PRLR analog containing one of the other single tyrosine displays drastically reduced or

even no activity in such bioassay (514). Furthermore, we have shown that the presence of the C-terminal tyrosine on both monomers of the dimerized PRLR complex is required to achieve a fully functional receptor on a PRL-responsive reporter gene (512). In good agreement, Clevenger and colleagues reported recently that mutation of Tyr 382 within the intracellular domain of a single PRLR (intermediate form) involved in a dimerized complex strongly decreases PRL-induced proliferation of BA/F3 cells (510a). In contrast, however, point-mutation of Tyr 580 in the long PRLR isoform has only a partial effect on PRLR function (513), whereas the absence of this residue in bovine and cervine tissues that naturally lack this C-terminal tyrosine compared with other species is not detrimental to receptor activity (523, 524), indicating that alternative intracellular tyrosines can be used. For example, a recent study has suggested an important role of Tyr 309 in the transcriptional activation of the IRF-1 promoter (507), most probably through Stat1 activation (see below). Similarly, dimerization of GM-CSFR/PRLR chimeras in which a single cytoplasmic domain lacks Tyr 309 only achieves partial activation of JAK2 and Fyn kinases and reduced ligand-induced proliferation of BA/F3 cells (510a). Tyrosine 309, which in our bioassays was not critical for β-casein gene activation (513), might thus be required for some biological functions and/or in some cell types.

Why is receptor tyrosine phosphorylation required? Although available data remain limited, it appears that PRLR-mediated cell proliferation does not necessarily require tyrosine phosphorylation of the receptor. For example, a mutant form of the long PRLR truncated after 94 cytoplasmic residues (G328) maintains the ability to activate Stats 1, 3, and 5 and to transduce a significant proliferative signal in murine 32D cells, presumably through signaling pathways involving these Stat molecules (see below) (437). Although the phosphorylation status of this G328 mutant was not established by the authors (437), a mutational study of tyrosines in the intermediate PRLR (513) suggests that this G328 mutant is not phosphorylated, although phosphorylation of tyrosine 309 cannot be totally discarded (514). These results are in agreement with the observation that the nonphosphorylated short PRLR can induce proliferation of NIH-3T3 fibroblasts (525). However, some contradiction exists since the short PRLR was otherwise reported to be unable to stimulate the proliferation of FDC-P1 and Ba/F3 cells (526), as is a homodimerized intermediate PRLR truncated at residue 322 (thus containing a longer tail than the short isoform) expressed in Ba/F3 cells (510a). Such discrepancies might reflect some differences inherent to the cell types (*e.g.*, fibroblastic *vs.* hematopoietic) that were used for these experiments. It thus appears premature to deduce any general rule with respect to PRLR domains required for proliferation. Beyond receptor phosphorylation, it is particularly interesting to note that heterodimerization of wild-type rat PRLR cytoplasmic domains (short/long, short/intermediate, long/intermediate) fails to induce BA/F3 cell proliferation (510a). Since tissues expressing the PRLR presumably express more than a single isoform, this observation might be of considerable functional significance and represents an open area of investigation.

In contrast to cell proliferation, transactivation of reporter genes (luciferase/chloramphenicol acetyltransferase) controlled by promoters of PRL target genes (*e.g.*, milk proteins such as β-casein) requires additional C-terminal region(s) of the receptors, most probably for the presence of phosphorylated tyrosines within these domains. For example, the rat short PRLR (501), as well as the nonphosphorylated Nb2 mutant lacking the C-terminal tyrosine 382 (513), are unable to stimulate the β-casein promoter and both function as dominant negatives of the long form, strengthening the likelihood that functional complexes require a phosphorylated tyrosine on both receptors forming the homodimer (512). Activation of the IRF-1 promoter, a growth-related PRLR-target gene (441, 527), depends on both tyrosines 309 and 382 of the intermediate PRLR, and it has been suggested that these tyrosines, when phosphorylated, may serve as docking sites for recruiting Stat1 (482, 507). However, we have shown that a mutant form of the long PRLR deleted of all its cytoplasmic tyrosines maintains the ability to transmit a (weak) lactogenic signal in the particular context of JAK2 overexpression, suggesting that the kinase can partially initiate PRLR signaling pathways (514). In agreement, it has been suggested that JAKs by themselves are capable of generating a signal and activating promoters in the absence of a specific ligand-receptor interaction, correlating the ability of the kinase to phosphorylate Stat5 *in vitro* (528).

In conclusion, although current data do not unambiguously establish receptor phosphotyrosines as an absolute requirement for the activation of PRLR target genes, they undoubtedly enhance the signal of PRL on these genes.

3. Stat proteins.

Stat family. Stat is a family of latent cytoplasmic proteins of ~90–100 kDa identified within the past 4 yr on the basis of their involvement in cytokine receptor signaling. The Stat gene family currently contains eight members: Stat1 (α and β), Stat2, Stat3, Stat4, Stat5a, Stat5b, Stat6 (or IL-4 Stat), and dStat, a Stat5 homolog found in *Drosophila*. Stats contain five conserved features: a DNA-binding domain, a SH3-like domain, a SH2 domain, a ubiquitous tyrosine and a C-terminal transactivating domain (from the N- to C-terminus, respectively). A consensus model of Stat activation has been proposed on the basis of data collected from studies of the different cytokine receptors (for additional information, see previously published reviews on Stat proteins, Refs. 44, 495, 529–532). First, the cytokine-bound receptor undergoes tyrosine phosphorylation by the associated Janus kinase. Second, the phosphorylated tyrosine interacts with the SH2 domain of a Stat, making the latter a part of the receptor-JAK complex. Third, the receptor-bound Stat is phosphorylated by the Janus kinases belonging to the complex. Fourth, the phosphorylated Stat dissociates from the receptor, homo- or heterodimerizes through an interaction involving the phosphotyrosine of each monomer and the SH2 domain of the other Stat molecule, and finally the dimer translocates to the nucleus where it activates specific DNA elements found in the promoters of cytokine target genes.

Stats involved in PRLR signaling. The specificity of a cytokine receptor is believed to be driven, at least in part, by the number and type of Stat proteins it can activate. Three mem-

bers of the Stat family have been thus far identified as transducer molecules of the PRLR: Stat1, Stat3 and, mainly, Stat5.

In 1994, the transcription factor named MGF (mammary gland factor) was identified from sheep mammary gland (533) and, in view of the high structural similarity with the other Stat proteins known at that time, it was further renamed Stat5 (528, 530). Within the past 3 yr, Stat5 cloning from mouse (534–536), rat (537), and human (538, 539) revealed the existence of two genes (Stat5a and Stat5b) encoding several isoforms that show 90–95% similarity, the major differences lying within the C-terminal domain. All isoforms possess the functionally essential tyrosine 694, identified by Gouilleux and collaborators (528) as the tyrosine being phosphorylated by JAK2. In agreement, PRLR mutants not able to associate/activate Janus kinases are unable to activate Stat5 (508, 514). Similarly, when the C-terminal domain of Stat5 is truncated (which removes the tyrosine phosphorylation site), such mutants function as a dominant-negative (540–542). Finally, it is noteworthy that in the specific context of cytokine chimeric receptors, JAK1 and possibly JAK3 can also phosphorylate Stat5 on tyrosine (543).

In agreement with the consensus model of Stat recruitment by receptor phosphotyrosines, we have shown that in the long PRLR, tyrosines 580, 479, and 473 (which are all phosphorylated in the context of single-tyrosine mutants), are sufficient to activate Stat5, although Tyr 580 is clearly the most potent in this respect (514). Similarly, mutation of the C-terminal tyrosine 382 in the intermediate PRLR, which abolishes tyrosine phosphorylation of the receptor (see above), prevents PRLR-mediated activation of the β-casein promoter (513). These data strongly suggest that in both long and intermediate PRLR isoforms, this particular tyrosine residue is involved in Stat5 recruitment and/or activation (Fig. 2B). In contrast, others have reported that a C-terminal truncated form of the long PRLR (G328, lacking Tyr 382) is able to stimulate Stat5 tyrosine phosphorylation (508), although the transcriptional activity of this mutant on a PRL-responsive reporter gene was not assessed. Others have shown that the C-terminal tail of the rabbit PRLR is not an absolute requirement, but considerably amplifies the transcriptional activation of PRLR-sensitive genes such as the β-lactoglobulin (500). Although these apparent discrepancies might reflect species specificity (of cellular systems used for bioassays, or of PRLR), they might also indicate that Stat5 tyrosine phosphorylation is required (528), but not necessarily sufficient for activation of transcriptional activity. Accordingly, it has been shown recently that serine/threonine phosphorylation is an absolute requirement for transcriptional activation of Stat5 by the IL-2R (544). Interestingly, Stat1, Stat3, Stat4, and sheep Stat5 contain potential MAP kinase serine phosphorylation sites (P-x-S-P) in their C termini (545). In human, mouse, and rat Stat5a, but not Stat5b, a very similar tetrapeptide is found at the homologous position (R-L-S-P). Although this sequence does not perfectly match the MAP kinase consensus site, it might be a target for another proline-directed kinase (539). Beadling and colleagues (544) have shown that IL-2-induced activation of Stat5 is not mediated by the ERK2/MAP kinase pathway. PKCα and casein kinase II have been proposed as candidates for serine phosphorylation of Stat5 (537, 544). Such a hypothesis may correlate with the previously reported implication of both kinases in the regulation of the β-casein gene by PRL (546, 547). Although functional distinction between Stat5a and Stat5b remains to be investigated in detail, it might be partly correlated with these putative serine/threonine phosphorylation sites. In this respect, heterodimerization of Stat5a and Stat5b has been recently reported by several investigators (548–550). Moreover, Kirken *et al.* (550) have recently shown first that PRL induced phosphorylation of both Stat5a/b on serine, but not on threonine in Nb2 cells, and second that the kinetics of this phosphorylation were markedly different for both Stats (550). Whether this observation reflects any functional difference between these two closely related transcription factors awaits further investigation. Finally, Yu-Lee and colleagues (482, 507, 551) have shown recently that Stat5a and Stat5b exert an inhibitory effect on PRL-inducible IRF-1 promoter activity, and these authors have proposed this inhibition to involve squelching by Stat5 of a factor that Stat1 requires to stimulate the IRF-1 promoter.

In addition to Stat5, Stat1 and Stat3 both have been reported to be activated by the PRLR (436, 501, 508). Stat3, also named APRF (acute phase response factor), was cloned as an IL-6-activated transcription factor (552). Stat1 was first isolated as part of the ISGF3 complex (containing Stat2 and a DNA-binding protein called p48) which is typically activated by IFNs α and β (553). Stat1 homodimers can also be formed upon IFNγ activation. The region(s) of the PRLR required for activation of these Stats remain poorly documented, although the 93 membrane-proximal residues have been reported to be sufficient for tyrosine phosphorylation of both Stats 1 and 3 (508). In the context of the GHR, it has recently been hypothesized that phosphotyrosine(s) of JAK2 could also bind to Stat3, in agreement with the presence of the consensus Stat3-binding site (see below) in the kinase (516, 520, 554, 555). Although such an interaction does not preclude the possible occurrence of interactions also with the receptor, this hypothesis remains to be tested in the context of Stat activation by the PRLR.

Stat-binding phosphotyrosine motifs in the PRLR. As described above, within a given receptor cytoplasmic domain, not all tyrosines undergo phosphorylation upon ligand-mediated receptor activation. The molecular features directing one tyrosine, and not another, to become phosphorylated are still poorly understood. Obviously, one requirement is the accessibility of the residue by the kinase. A phosphotyrosine must be recognized by at least three types of signaling proteins: the tyrosine kinase, the tyrosine phosphatase that will down-regulate the signal, and the SH2- (or any other motif recognizing phosphotyrosine) containing protein(s) that will dock on the phosphotyrosine. It is usually accepted that some of these interactions are driven by the recognition of specific sequences surrounding the phosphotyrosine (556, 557). Attempts have thus been made to correlate the ability of cytokine receptors to bind a given subset of Stat proteins with the presence of consensus sequences in the near environment of their cytoplasmic phosphotyrosines (520, 558–561).

Regarding Stat5 recruitment by the long PRLR, no sequence similarity could be found around the three phosphorylated tyrosines involved (Tyr 580, Tyr 479, Tyr 473; see above and Fig. 2B). The D-x-Y motif, involving some ty-

rosines within the GHR (516, 520), EPOR (548, 562, 563), and IL-2Rβ (544, 564), has been proposed as a putative Stat5-binding motif (520); none of the three phosphorylated tyrosines of the PRLR (514) matches such a consensus sequence. Recently, it has been suggested that an Asp at position −2 or −1, and a hydrophobic residue (preferentially a Leu or any aliphatic side chain) at positions +1 and +3 with respect to the phosphotyrosine could favor Stat 5 binding (561). Interestingly, two of the three proposed Stat5-binding sites in PRLR (Tyr 580 and 479) have an Asp at position −1, and all (including Tyr 473) have a hydrophobic residue at position +1 (Leu or Val). In contrast, the three other phosphotyrosines, which do not lead to Stat 5 activation, *i.e.*, Tyr 309, 402, and 515 (514) are in less agreement with this proposed consensus (Fig. 2B). In fact, the sequence bordering Tyr 402 is more closely related to that described for insulin receptor substrate-1 (IRS-1) binding.

Finally, we note the sequence bordering Tyr 309, which is phosphorylated in the context of a single-tyrosine PRLR mutant (514), matches the Y-x-x-Q sequence identified as Stat3-binding motif (558) and closely resembles that described for Stat1 binding (Y-P-x-Q instead of Y-x-P-Q; Fig. 2B) (559). This residue could thus be involved in Stat3 and/or Stat1 recruitment, in agreement with data obtained using a PRLR truncated at residue 328 (508). A similar proposal has been formulated recently by Yu-Lee and colleagues (482, 507) in view of the decrease of Stat1-mediated PRL induction of IRF-1 promoter activity. As an alternative to Stat/receptor direct interactions, such a consensus sequence is also present in JAK2 (555, 565).

Stat DNA-binding motifs. Consensus DNA motifs specifically recognized by Stat complexes have been identified in the promoters of target genes. The motif termed GAS (for γ-IFN-activated sequence) was defined using Stat homodimers and consists of a palindromic sequence TTC xxx GAA (532, 566). The specificity of the interaction between a particular Stat and a GAS motif found in a given target promoter has been proposed to depend, at least in part, on the center core nucleotide(s) (532). The activation of identical Stat proteins by different cytokine receptors questions the mechanisms by which specificity of signaling pathways is achieved in response to a particular hormonal stimulation. Although several cytokines (EPO, GM-CSF, GH, PRL, IL-2, IL-3, IL-5) activate the DNA-binding ability of Stat5 and/or transactivate the β-casein luciferase reporter gene *in vitro* (516, 536, 543, 564, 567–571), it is unlikely that all these cytokines stimulate the synthesis of this milk protein *in vivo*. This suggests that different Stat combinations and/or involvement of other signal transducers direct the specificity of the final response. For example, it has recently been reported that Stat5 interacts with the glucocorticoid receptor (572). This functional cooperation seems to require specific DNA binding of Stat5, but not of the glucocorticoid receptor (572a), although this hypothesis remains controversial (573).

In conclusion, particular emphasis must be given to the activation of Stat5, which is probably the major axis of the JAK-Stat cascade, if not of all signaling pathways activated by the PRLR, as confirmed by the very similar phenotypes analyzed thus far in PRLR, Stat5a and Stat5b knockout mice (see *Section VII*).

B. The Ras/Raf/MAP kinase pathway

Although the JAK-Stat cascade is presumably the most important signaling pathway used by cytokine receptors, other transducing pathways are also likely involved in signal transduction by these receptors. Signaling through MAP kinases (MAPK) involves the Shc/SOS/Grb2/Ras/Raf/MAPK cascade (574). Activation of the MAPK pathway has been reported in different cellular systems under PRL stimulation (157, 208, 443–445, 447, 525, 575). Whether activation of the MAP cascade requires JAK2, Fyn (or any Src kinase; see below), or any other pathway is currently unknown. Activation of the nucleotide exchange protein Vav has also been reported (446). Although the JAK-Stat and the MAPK cascades were initially regarded as independent pathways, several recent data suggest rather that they are interconnected (576).

C. Other signaling pathways

Fyn, a member of the Src kinase family (577), is associated with the PRLR and is activated by PRL stimulation in the rat T lymphoma Nb2 cell line (440). Association of the PRLR with Src, the prototype member of this kinase family, has also been reported after PRL stimulation in lactating rat hepatocytes (162). The role of Src kinases in signal transduction by PRLRs remains unknown, although promotion of cell growth has been suggested (162).

Using a modified rat PRLR cDNA encoding an additional N-terminal epitope specifically designed to allow the rapid purification of the receptor and associated proteins from transfected cells, we have recently shown that PRL induces a rapid tyrosine phosphorylation of IRS-1 and of the 85-kDa subunit of the phosphatidyl-inositol (PI)-3′ kinase (450, 455). Both PI-3′ kinase and IRS-1 appear to associate with the PRLR in a PRL-dependent manner. Recently, it has been proposed that PRL activation of PI-3′ kinase might be mediated by Fyn in Nb2 cells (577a).

Since most transducer molecules are activated by tyrosine phosphorylation (JAKs, Stats, Src, etc.), involvement of tyrosine phosphatases to modulate or down-regulate the signaling cascades is expected. Accordingly, several recent studies pointed out a role of tyrosine phosphatases in PRLR signaling (451, 578, 579), although the mechanism by which they are regulated, as well as their substrates, remain poorly documented. We have reported that the phosphatase PTP-1D (now renamed SHP-2; see Ref. 580), is activated by JAK2 and acts as a positive regulator of PRLR-dependent induction of β-casein gene transcription (451). Moreover, a new family of SH2-containing protein inhibiting the JAK/Stat pathways has been recently identified (580a–580d). Members of this new protein family were named CIS (cytokine-inducible SH2-containing protein), SOCS (suppressor of cytokine signaling), JAB (JAK binding protein) and SSI (Stat-induced Stat inhibitor), and they inhibit the JAK/Stat pathway by interacting with JAKs (SOCS) or by competing with Stats for binding to the receptor (CIS). These proteins appear as targets of activated Stats and hence, provide a way to down-regulate the JAK/Stat pathways. PIAS3 (protein inhibitor of activated Stat) directly interacts with Stat3 and blocks its

binding to DNA targets (580e). Although involvement of these negative regulators of cytokine-activated JAK/Stat pathways has not been reported in connection with the PRLR, it is likely that at least some of these proteins are involved in negative feedback control of PRLR signaling.

Involvement of phospholipase-C (PLCγ) and PKC has been suggested, although the role of these enzymes in PRLR signaling remains unknown and their substrates remain poorly identified (155, 176, 177, 581). Finally, PRL has been shown to increase the concentration of intracellular calcium in PRLR-transfected CHO cells, although the physiological meaning of this phenomenon remains to be elucidated (582).

Cross-linking experiments have suggested that the PRLR is complexed to G proteins in Nb2 cells (583). The same group proposed the involvement of these guanine nucleotide-binding proteins in the mitogenic action of PRL on Nb2 cells (584). A potentially new signal transducer has been recently identified in rat ovary and appears to be specific to this tissue (585). This protein, named PRAP (PRLR-associated protein), preferentially binds to the short PRLR isoform and has been proposed to contribute to the luteotropic effects of PRL in the corpus luteum.

Finally, cross-talk between PRLR and EGF receptor (EGFR) has been recently suggested (585a, 585b). PRL could antagonize EGF signaling by increasing EGFR threonine phosphorylation and thereby decreasing EGF-induced EGFR tyrosine phosphorylation.

VII. Null Mutation of the PRLR Gene

Almost all of the effects attributed to PRL in vertebrates (92, 586) are the subject of conflicting observations and despite an amazing accumulation of articles, the precise role of lactogenic hormones, with the clear exception of direct mammary effects, remains an open question. Lactogens thus remain well characterized hormones with multiple but, in many cases, not well characterized functions.

In vivo, two animal models have been developed to study PRL action: one utilizes lowering PRL levels, achieved either by hypophysectomy or administration of dopamine D_2 receptor agonists; the second is based on the use of spontaneous mutant dwarf mice strains, lacking pituitary somatotrophs and lactotrophs. These models are, however, severely compromised by incomplete PRL depletion (extrapituitary PRL is not affected in these models; see *Section II.D*), and by the unavoidable suppression of other pituitary and nonpituitary hormones. *In vitro* models suffer similar limitations. FCS, an essential component of most cell and tissue culture media, contains high concentrations of lactogenic hormones that are difficult to remove (207). In addition, a growing list of cells (see *Section II.D*) synthesize and secrete PRL (27, 587, 588), making complete PRL removal impossible.

By the technique of gene targeting in mice (589), we have produced the first experimental model in which the effects of a complete absence of PRLR-mediated signaling of lactogenic hormones can be observed (214). Cell lines derived from this particular mouse offer the potential of *in vitro* models to further explore the indirect and direct effects of PRL. The vast majority of mutations created by homologous recombination in embryonic stem cells to date have been null alleles. The next level of sophistication requires that a gene be inactivated or modified in specific tissues or at a certain time during the life of the animal (conditional knockouts or knockins).

The PRLR is expressed as short and long forms, differing in the length and sequence of their cytoplasmic tails (*Section III.B*; see Refs. 33, 46, 48, 50, and 51). The short and long forms are differentially expressed or regulated during the estrous cycle and pregnancy (52, 590, 591), which suggests that they may initiate distinct signaling pathways.

A. Gene cloning, vector construction, and generation of $PRLR^{-/-}$ mice

A 129Sv mouse genomic DNA library was screened using the mouse PRLRS3 cDNA (51), and the coding region of the PRLR gene was isolated. Loss of just one cysteine of exons 4 or 5 encoding extracellular subdomain D1 results in complete lack of hormone-binding activity (66). A targeting construct was prepared with 7.5 kb of overall homology in which a 1.5-kb fragment containing exon 5 was replaced with the similarly sized Tk-NEO cassette, which resulted in a mutation creating an in-frame stop codon. After electroporation into E14.1 embryonic stem cells and neomycin selection, two selected clones were microinjected into 3.5-day-old C57BL/6 blastocysts and were able to generate germline chimeras. F1 intercrosses revealed a genotype distribution not significantly different from the normal Mendelian ratios excluding the first generation (214).

B. PRLR gene expression and PRLR protein in $PRLR^{-/-}$ mice

By Northern blotting, a single major mRNA transcript of 2.8 kb was observed in $PRLR^{+/+}$ but not in $PRLR^{-/-}$ animals, demonstrating that no PRLR mRNA containing exon 5 is transcribed: the deletion of exon 5 was also confirmed by RT-PCR.

The PRLR protein was immunoprecipitated from solubilized liver microsomes and analyzed by immunoblot. A strong signal for the PRLR was detected using an antirat PRLR U5 (592) in the liver of $PRLR^{+/+}$ mice; however, no PRLR protein could be detected in the liver of $PRLR^{-/-}$ animals. $PRLR^{-/-}$ mice showed no significant specific binding of PRL in liver microsomes. All data indicate that the exon 5 deletion caused the complete absence of functional PRLR in $PRLR^{-/-}$ animals (214).

C. Impaired mammary gland development and lactation in heterozygous females

Most of the first litter of 6- to 8-week-old PRLR heterozygote ($PRLR^{+/-}$) F1 females died within 24 h, and the entire litter had perished by 48 h. The examination of the stomach contents of the pups showed air bubbles but no milk present, indicating that $PRLR^{+/-}$ females were unable to lactate. This phenotype was not apparent after the second pregnancy, where all F1 $PRLR^{+/-}$ females produced surviving pups. A similar phenotype was also seen at the first lactation in het-

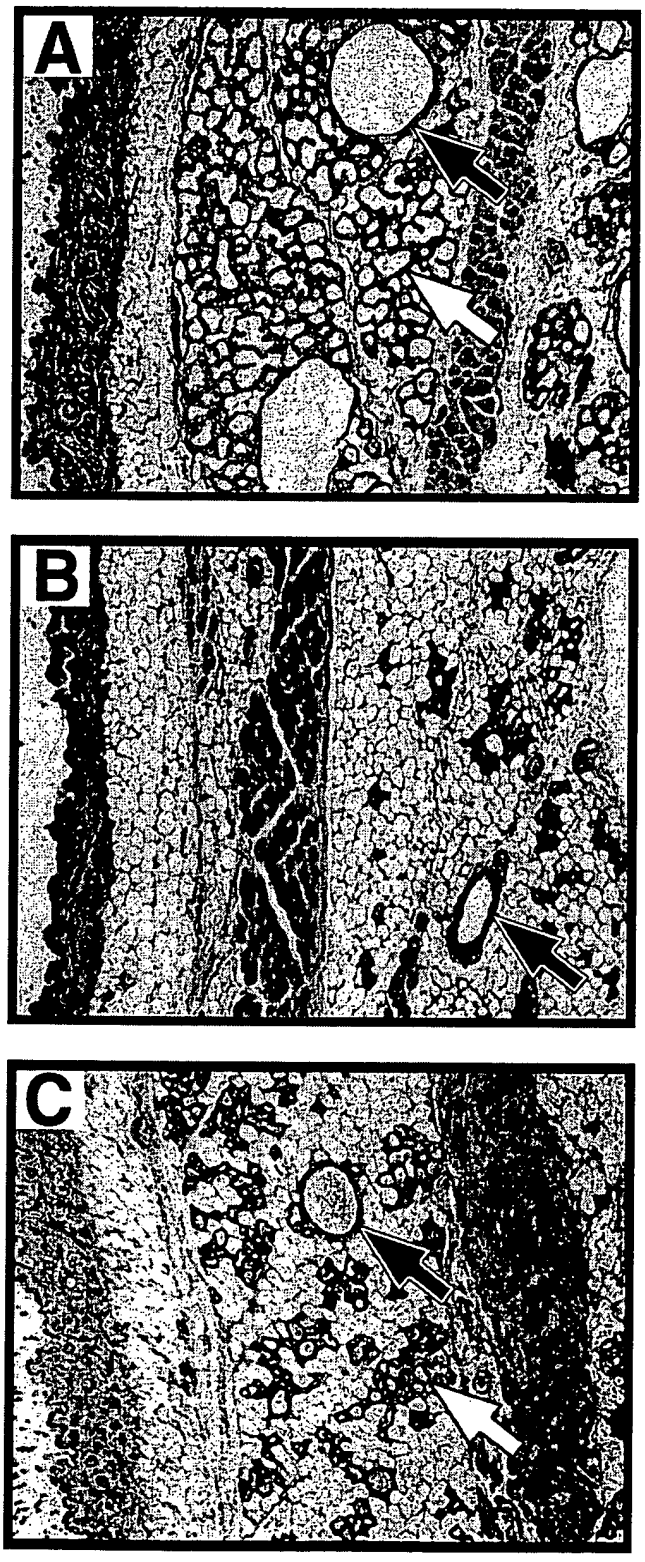

FIG. 4. Histology of second thoracic mammary glands from PRLR$^{+/+}$ and PRLR$^{+/-}$ females. Hematoxylin/eosin-stained sections through the skin (*left-hand side* of each picture), subcutaneous fat (light staining), mammary fad pad (light staining), and epithelium (dark staining) of the number 2 and 3 glands (magnification, 100×). Alveoli are indicated by *white arrows*, while ducts are indicated by *black arrows*. A, F1 PRLR$^{+/+}$ female 48 h post partum. Note engorged alveoli. B, F1 PRLR$^{+/-}$ female unable to lactate 48 h post partum. Note absence of alveoli and dominant ductal tissue. C, F1 PRLR$^{+/-}$ female showing partial lactation at 48 h post partum, with some functional alveoli present.

erozygous F2 females whatever the genetic backgrounds (C57BL/6 × 129 and 129 F1). However, the 20-week-old PRLR$^{+/-}$ sisters of these animals produced their first litter with all surviving pups.

Histological examination of the mammary glands showed that lactational performance was correlated with the degree of mammary gland development (Fig. 4). These results demonstrate that two functional alleles of the PRLR are required for efficient lactation and that this phenotype in heterozygotes is primarily due to a deficit in the degree of mammary gland development.

The mammary gland undergoes development *in utero*, at puberty (mainly ductal development), and during pregnancy (ductal and alveolar development). The essential hormonal factors regulating the later two phases in mice have been established to be estrogen, adrenocorticoids, and GH during puberty, and estrogen, progesterone, and PL and/or PRL during pregnancy (284, 593). These hormones produce some development with each estrous cycle and massive development at pregnancy, which never fully regresses after estrus or weaning, resulting in ever increasing alveolar and ductal development with each episode (594). Our observations suggest that the epithelial cell proliferation during pregnancy and the postpartum period depends on a threshold of PRLR expression which is not achieved with just one functional gene, given that the level of PRLR is closely controlled in mammary gland (595). In heterozygous mice where the level of the receptor is reduced, mammary gland proliferation is insufficient to ensure lactation at the first pregnancy but further estrous cycles or a single pregnancy lead to the development of a mammary gland capable of producing milk.

Initial histological investigation of the virgin gland of mature PRLR$^{-/-}$ animals indicated no dramatic differences as a result of the null mutation of the PRLR, with ductal tissue clearly present, confirming that the PRLR is not essential for this stage of development similar to what has been reported for the null mutation of the progesterone receptor (596). The effect of this mutation on mammary development during pregnancy will be analyzed by transplantation of PRLR$^{-/-}$ mammary epithelium to PRLR$^{+/+}$ mammary fat pads cleared of endogenous epithelial cells before puberty.

D. Heterozygote maternal behavior

Some PRLR$^{+/-}$ mothers of 6–8 weeks and 20 weeks of age were observed to scatter their pups throughout the cage, often burying them completely in sawdust. When the mother reformed the nest, a pup was often left outside and not retrieved, while the others were suckled. This behavior was never observed among PRLR$^{+/+}$ and multiparous PRLR$^{+/-}$ females. In addition, PRLR$^{+/-}$ females generally did not eat their dead pups, in contrast to PRLR$^{+/+}$ females.

Other processes regulated by the hypothalamus, such as

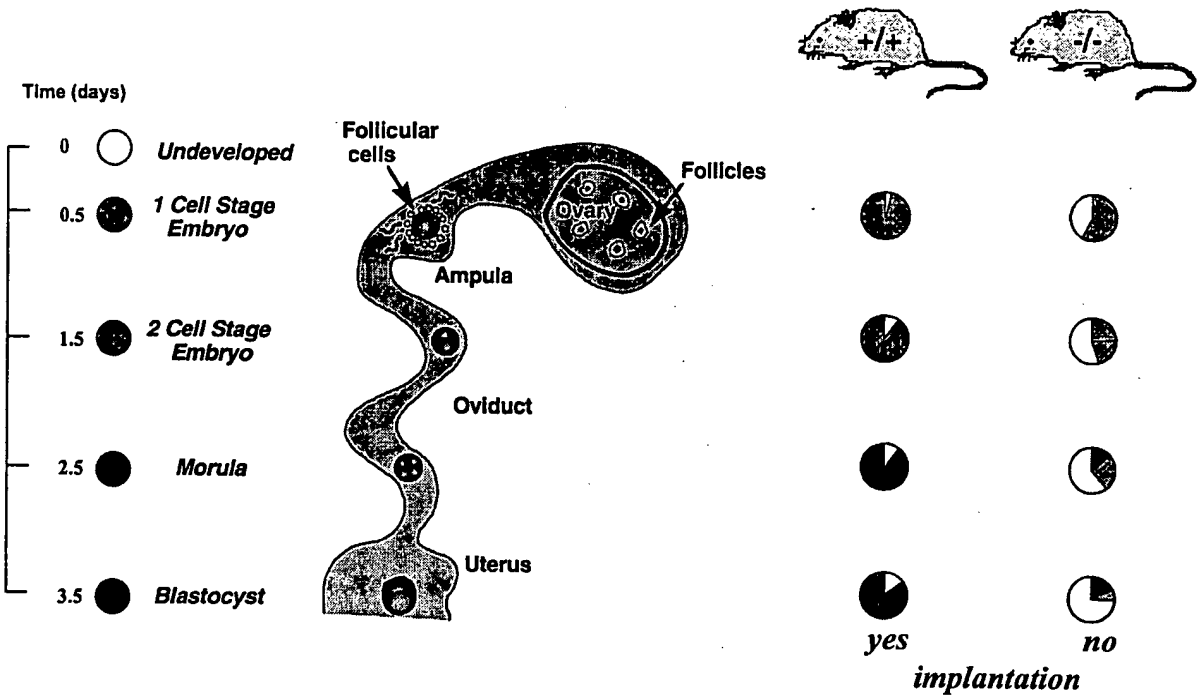

FIG. 5. Schematic representation of preimplantation egg development in PRLR$^{+/+}$and PRLR$^{-/-}$mice. On the left, normal egg development is represented. Undeveloped oocytes are depicted in white (including oocytes at the germinal vesicle stage, oocytes that have expulsed the first polar body, and degenerated embryos), and 1 cell and 2 cell embryos, morula and blastocysts are color-coded. On the right, pie charts of the various stages of egg development at different times after ovulation are represented in +/+ *vs.* −/− mice.

eating, sexual behavior, and locomotor activity, appear to be normal in PRLR mutant mice.

E. Homozygous females are sterile

The PRLR$^{-/-}$ females mated irregularly, every 3 to 4 days, and never became pregnant. They show a number of reproductive deficiencies: all PRLR$^{-/-}$ females were sterile despite mating, which did not produce a pseudopregnancy. This was confirmed by examination of estrogen levels which showed a marked increase on day 3 after the vaginal plug, as the animals again entered estrus. The irregular mating patterns of the females indicate an alteration of estrous cyclicity. After mating, the PRL surges initiated by mechanical stimulation of the cervix are thought to induce pseudopregnancy (597), and the observation that the PRLR$^{-/-}$ females remated every 3 to 4 days demonstrates that a functional PRLR is essential for the establishment of pseudopregnancy.

Multiple abnormalities were observed: fewer eggs were fertilized, oocytes at the germinal vesicle stage were released from the ovary, and fragmented embryos were found. The number of eggs ovulated was reduced, and histological investigation showed fewer primary follicles in PRLR$^{-/-}$ ovaries. Only 19% of blastocysts were recovered at day 3.5 in the uterus of PRLR$^{-/-}$ against 85% in wild-type animals (Fig. 5). Single cell-fertilized eggs were recovered, suggesting for most oocytes that an arrest of development occurred immediately after fertilization.

The PRLR$^{-/-}$ females were mated to PRLR$^{+/+}$ males, and fertilized embryos were reimplanted into the oviducts of pseudopregnant foster mothers. Most of the fertilized eggs produced normal embryos. Using fertile PRLR$^{-/-}$ males (to exclude the possible paternal contribution of PRLR to the embryo), embryos were also recovered and reached adulthood, demonstrating that the eggs are viable, and thus that lack of blastocyst implantation in PRLR$^{-/-}$ females may be due to a deficient environment in the oviduct.

Thus, the absence of PRLR in female mice results in reduced ovulation, reduced fertilization, and almost complete arrest of preimplantation development. The blastocysts were unable to implant in PRLR$^{-/-}$ females, indicating that the uterus of these animals is refractory to implantation. The outcome is complete sterility.

The high number of eggs still containing germinal vesicles that were ovulated in PRLR$^{-/-}$ animals indicates that the PRLR is important for oocyte maturation. Most of these were found on days 2.5 and 3.5 after the vaginal plug, suggesting that they may have been ovulated at a later time than those released at estrus; thus PRL may be involved in follicular atresia. A higher level of PRL is seen in follicles containing mature oocytes capable of being fertilized (337, 598–604), although others found no relationship (605) or suggest a negative effect (606). PRL was found to increase the rate of germinal vesicle breakdown and subsequent fertilization and correct development *in vitro* (607). The failure of a significant proportion of eggs to undergo germinal vesicle breakdown within maturing PRLR$^{-/-}$ follicles directly demonstrates the important influence of PRL and its receptor on oocyte maturation or atresia.

Fertilization rates were reduced, indicating that this effect is a result of a maternal deficiency. This may involve incom-

TABLE 8. Summary of phenotypes of $PRLR^{+/-}$ and $PRLR^{-/-}$ mice

	+/−	−/−
Reproduction		
Female	Normal mating, normal pregnancy	Sterility: No pseudopregnancy Ovulation rate decreased Oocyte maturation decreased No implantation
Male	Normal	Delayed fertility
Mammary gland development	Impaired development efficient lactation after the first pregnancy	No lactation: Ductal branching greatly reduced Absence of alveoli
Behavior		
Maternal	Reduced	Absent
Configural learning	Normal	Normal
Eating, locomotor activity, olfactory function	Normal	Normal
Immunity	Normal	No obvious differences

plete oocyte maturation, or a defect in the oviduct such as a reduced sperm transport to the ampulla or loss of a factor that enhances fertilization. Divergent effects of PRL on the rate of implantation development of mouse embryos have been reported (607–609). The present studies exclude the absolute requirement for an oocyte PRLR in pre- and postimplantation embryonic development, supporting previous investigations (610, 611) and indicating that the defect must reside in the environment in which the embryo develops. A number of factors in the oviduct that influence preimplantation development may be affected by the PRLR mutation. Estrogen and progesterone can influence the rate of ovum transport and preimplantation development (612).

The failure of trophic support of the corpus luteum by PRL would be expected to reduce progesterone levels on day 2.5 of pregnancy, when progesterone levels normally rise (613). Injection of antibodies against progesterone blocks mouse embryo development at the four-cell stage (614), but the failure of preimplantation development in $PRLR^{-/-}$ females occurs earlier, when progesterone levels are normal in $PRLR^{-/-}$ females. These results indicate that the PRLR must trigger a signal that occurs earlier than PRL-induced trophic support of the corpus luteum. Candidates include ovum factor, now identified as platelet activating factor (PAF), released by the fertilized eggs (615), and early pregnancy factor (EPF), a multifactorial activity comprised of PAF, thirodoxin (616), chaperonin 10 (617), and other uncharacterized molecules produced by the platelets of the oviduct and ovary in response to embryonic PAF (618). EPF is present in serum 24 h after ovulation and stimulates lymphocytes to produce a suppressor of the delayed-type hypersensitivity reaction, potentially protecting the ovum from the maternal immune system and promoting embryo cleavage, in addition to acting as a growth factor (619). Synthesis of EPF by isolated mouse oviducts and ovaries is stimulated by PRL, and PRL cooperates with PAF to stimulate ovarian EPF *in vivo* in response to fertilization, while passive immunization with antibodies against EPF produces almost identical effects as the PRLR mutation, with 54% of eggs not developing beyond the fertilized egg and two-cell stage (620–622).

Uterine preparation for embryo implantation is dependent upon continued estrogen and progesterone secretion by the corpus luteum of the ovary, which is supported by the pituitary in rodents during the first half of pregnancy (623). PRL has been shown to stimulate progesterone synthesis by dispersed ovarian cells from midpregnant mice (624), demonstrating that lactogenic hormones can directly stimulate ovarian progesterone secretion. Furthermore a nidatory ovarian estrogen surge is required to allow embryo implantation (625). Thus $PRLR^{-/-}$ females probably cannot support the implantation of blastocysts because the corpus luteum does not receive pituitary PRL support, and thus progesterone- and estrogen-dependent signals for implantation cannot occur. Recently, multiple reproductive failures similar to those observed in PRLR knockout mice have been reported in cyclooxygenase 2-deficient mice (625a).

F. Homozygous male fertility

The fertility of $PRLR^{-/-}$ males was examined by housing each male separately with a 12- to 14-week old $PRLR^{+/+}$ female of proven fertility. At estrus, all females showed vaginal plugs, indicating normal mating, intromission, and ejaculation, but 20% of all the tested males showed a delayed fertility. Testes and accessory organs were of normal size; their histological examination showed no obvious morphological or histological abnormalities: clearly defined germinal cell layers and spermatocytes were present in the seminiferous tubules.

PRL may regulate testosterone production by Leydig cells via modulation of the effects of LH and of the level of its receptor (35). PRL has been also proposed to be involved in sperm capacitation (626): a short period of incubation with PRL has been reported to enhance *in vitro* fertilization rates (369), while longer periods reduce *in vitro* fertilization rates (610, 627), although others have seen no effect (611). PRL can also influence the function of the accessory reproductive glands (391, 628). The fact that if one waits long enough, all $PRLR^{-/-}$ males are fully fertile, indicates that this role of PRL can be performed by other regulatory factors.

G. Other gene-targeted mutations leading to impaired mammary gland and reproductive functions

Although no effect on the mammary gland of heterozygous animals has been reported for null mutations of the progesterone receptor gene (596), the complete absence of this receptor in homozygous animals results in a gland lacking terminal-end buds with some branched ducts (596). Although estradiol receptor knockout females are infertile, to our knowledge, no particular mammary gland phenotype has been reported (629).

In Stat5a-deficient mice, mammary lobuloalveolar outgrowth during pregnancy was curtailed, and females were unable to lactate after parturition because of a failure of terminal differentiation (630). Similarly, mammary gland development is also impaired in Stat5b$^{-/-}$ females and, although milk protein genes are expressed, there is insufficient milk to feed pups (630a). Similar phenotypes have been recently reported in PRL knockout mice (631). Interestingly, Stat5b, but not Stat5a-deficient females exhibit severely compromised fertility. Moreover, the phenotype of Stat5b knockout mice is distinct from that of Stat5a-deficient mice by a decrease in body growth profile in the former.

Mice homozygous for a germline mutation in A-*myb*, a nuclear protein regulator of transcription, show a marked underdevelopment of the breast epithelial compartment after pregnancy. Consequently, mice are unable to nurse their newborn pups, demonstrating a critical role of A-*myb* in mammary gland development (632). Mice lacking the cyclin D1 gene also exhibit a dramatic impairment of mammary gland development leading to inability to lactate their litters (633).

In conclusion, the phenotypes of animals lacking functional genes encoding PRLR, PRL, Stat5a, or Stat5b confirm the essential role of these molecules in the signaling pathway(s) leading to mammary gland development and/or reproductive function, whereas estradiol and progesterone receptors are probably involved to a lesser extent in mammary gland development.

H. Other phenotypes of PRLR$^{-/-}$ mice

Immunoregulation. In view of the putative immunomodulatory role of PRL, we are currently analyzing the immune phenotype of the knockout animals. Preliminary data suggest that maturation and export of precursor cells occur in the thymus and, to date, no abnormality in the export of the lymphoid system to the periphery has been identified. Interestingly, no immune phenotype was seen in mice lacking the PRL gene (631). Specific functional studies to characterize individual immune cell types in PRLR$^{-/-}$ are ongoing.

Bone. The PRLR mRNA expression has been detected in murine bone cells. The level of the mRNA encoding the long form of PRLR in osteoblasts is comparable to that observed in other cells such as thymocytes, mammary cells, or bone marrow cells. No expression of any form was found in osteoclast-like cells. In initial studies, examination of the calvariae of PRLR$^{-/-}$ embryos at 18.5 days of age indicates that these bones are less developed or more disorganized than in wild-type controls.

Late fetal or neonatal lethality was the expected phenotype of PRLR$^{-/-}$ animals. PRLR expression rises dramatically in a number of rodent tissues during the late stages of pregnancy, and PRL and PLs are detectable in fetal blood (634, 635), suggesting that lactogenic hormones begin to exert major effects during this period in preparation for the transfer to autonomous life. In contrast, PRLR$^{-/-}$ animals were born and survived until adulthood. A number of phenotypes were observed in both hetero- and homozygous animals (see Table 8). It is probable that most of the phenotypes observed are related to the absence of the long form of the receptor, since this is the major isoform in all cells involved in reproductive function. Almost every aspect of female reproduction is altered in these animals, unambiguously demonstrating that the PRLR is a key regulator of reproduction. The ability of this new model to provide novel insights into the function of lactogenic hormones and their receptor illustrates the power of the knockout approach to discover and confirm specific roles for well investigated molecules. The generation of knockouts has highlighted the role of many genes in embryonic development, yet the study of phenotypes associated with null alleles is only an initial step in the analysis of the gene function. We can look forward, in the near future, to the widespread application of approaches of greater technical sophistication, including the generation of subtle alterations in the gene sequence and conditional knockouts.

VIII. Summary and Conclusions

PRL is an anterior pituitary hormone that, along with GH and PLs, forms a family of hormones that probably resulted from the duplication of an ancestral gene. The PRLR is also a member of a larger family, known as the cytokine class-1 receptor superfamily, which currently has more than 20 different members. PRLRs or binding sites are widely distributed throughout the body. In fact, it is difficult to find a tissue that does not express any PRLR mRNA or protein. In agreement with this wide distribution of receptors is the fact that now more than 300 separate actions of PRL have been reported in various vertebrates, including effects on water and salt balance, growth and development, endocrinology and metabolism, brain and behavior, reproduction, and immune regulation and protection. Clearly, a large proportion of these actions are directly or indirectly associated with the process of reproduction, including many behavioral effects. PRL is also becoming well known as an important regulator of immune function. A number of disease states, including the growth of different forms of cancer as well as various autoimmune diseases, appear to be related to an overproduction of PRL, which may act in an endocrine, autocrine, or paracrine manner, or via an increased sensitivity to the hormone.

The first step in the mechanism of action of PRL is the binding to a cell surface receptor. The ligand binds in a two-step process in which site 1 on PRL binds to one receptor molecule, after which a second receptor molecule binds to site 2 on the hormone, forming a homodimer consisting of one molecule of PRL and two molecules of receptor. The PRLR contains no intrinsic tyrosine kinase cytoplasmic do-

main but associates with a cytoplasmic tyrosine kinase, JAK2. Dimerization of the receptor induces tyrosine phosphorylation and activation of the JAK kinase followed by phosphorylation of the receptor. Other receptor-associated kinases of the Src family have also been shown to be activated by PRL. One major pathway of signaling involves phosphorylation of cytoplasmic Stat proteins, which themselves dimerize and translocate to nucleus and bind to specific promoter elements on PRL-responsive genes. In addition, the Ras/Raf/MAP kinase pathway is also activated by PRL and may be involved in the proliferative effects of the hormone. Finally, a number of other potential mediators have been identified, including IRS-1, PI-3 kinase, SHP-2, PLCγ, PKC, and intracellular Ca^{2+}.

The technique of gene targeting in mice has been used to develop the first experimental model in which the effects of the complete absence of any lactogen or PRL-mediated effects can be studied. Heterozygous (+/−) females show almost complete failure to lactate after the first, but not subsequent, pregnancies. Homozygous (−/−) females are infertile due to multiple reproductive abnormalities, including ovulation of premeiotic oocytes, reduced fertilization of oocytes, reduced preimplantation oocyte development, lack of embryo implantation, and the absence of pseudopregnancy. Twenty per cent of the homozygous males showed delayed fertility. Other phenotypes, including effects on the immune system and bone, are currently being examined.

It is clear that there are multiple actions associated with PRL. It will be important to correlate known effects with local production of PRL to differentiate classic endocrine from autocrine/paracrine effects. The fact that extrapituitary PRL can, under some circumstances, compensate for pituitary PRL raises the interesting possibility that there may be effects of PRL other than those originally observed in hypophysectomized rats. The PRLR knockout mouse model should be an interesting system by which to look for effects activated only by PRL or other lactogenic hormones. On the other hand, many of the effects reported in this review may be shared with other hormones, cytokines, or growth factors and thus will be more difficult to study.

Although PRL evolved several hundred million years ago, we are now at the end of the 20th century just beginning to understand how the hormone acts and its potential involvement in pathological disease states. Future research will center on further expanding the already long list of PRL actions and attempt to better understand the mechanisms of action of this intriguing hormone.

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